A

(i)

Copy Number	0	1	2	3	mean	Total cells
Test 1	10	35	209	0	1.78	254
Test 2	13	50	220	20	1.82	303

(ii)

B (i)

(ii)

C (i)

(ii)

D (i) Normal human genome

AAATTGTGCTA**CATATAT**ACCATGGGAT

(ii) TL-Om1 genome

HTLV-1 provirus

AAATTGTGCTA**CATATAT**   **CATATAT**ACCATGGGAT

NT_077389 164570   **NT_077389 164576**

**Underlined repeated sequence: NT_077389, 164570-164576**

FIG 2 Clonality, copy number, and integration site of HTLV-1 in TL-Om1 cells. (A) HTLV-1 proviral copy number per cell was determined by FISH using an HTLV-1 full-genome probe. (i) Yellow signals indicate the HTLV-1 probe. Lower table shows the counts of HTLV-1 signals per cell. (ii) Vertical axis indicates percentage counts of each fraction in relation to total cells. Data were the results from two independent analyses. (B) Number of HTLV-1 integrated chromosomes was determined in metaphase cells with the HTLV-1 and 1q44 probes. (i, ii) Yellow signals indicate the HTLV-1 probe, and red signals indicate the 1q44 probe. All HTLV-1 signals were located on chromosome 1. HTLV-1 signals on chromosome 1 were positioned at 1p13. (C) Determination of the HTLV-1 integration site in TL-Om1 cells. (i) The 3′ integration site was determined by Splinkerette PCR with an HTLV-1-specific primer. PCR products were subjected to agarose gel electrophoresis. (ii) BamHI- or XbaI-digested TL-Om1 genomes were self-ligated and subjected to inverse PCR with an HTLV-1-specific primer set. PCR products were subjected to agarose gel electrophoresis. (D) 5′ and 3′ HTLV-1 integration sites were determined by a sequencing analysis of DNA fragments from both Splinkerette and inverse PCR. (i) Normal human sequence; (ii) determined HTLV-1 integration site. HTLV-1 was inversely integrated at chromosome 1: NT_077389, 164570 to 164576.

value of target gene primers from the average C_T value of RPPH1 primers (Table 1). The correction value was calculated as follows: correction C_T value = correction factor × actual C_T value. By applying the correction factors, we reduced the limits of error of the C_T values to 0.1 cycles with normal PBMCs (data not shown).

Digital PCR analysis. Primers and probes for digital PCR analysis of HTLV-1 were previously reported (11, 15). In brief, the primers and probe for HTLV-1 were as follows: forward, 5′-CGGATACCCAGTCTACGTG TT-3′; reverse, 5′-CAGTAGGGCGTGACGATGTA-3′; probe, FAM-5′-CTGTGTACAAGGCGACTGGTGCC-3′-TAMRA (where FAM is 6-car-

boxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine). The primers and probe for albumin were as follows: forward, 5′-TGTCAT CTCTTGTGGGCTGT-3′; reverse, 5′-GGTTCTCTTTCACTGACATC TGC-3′; probe, FAM-5′-CCTGTCATGCCCACACAAATCTCTCC-3′-TAMRA. The mixture of primers and probe for RPPH1 was purchased from Applied Biosystems. The PCR mixture was prepared using 2× digital droplet PCR (ddPCR) supermix for probes (Bio-Rad, Hercules, CA, USA). Droplets were prepared on a QX100 droplet generator (Bio-Rad). PCR was performed with a LifePro thermal cycler (Bio-Rad) and detected with a QX100 droplet reader (Bio-Rad). Data were means of triplicate analysis.

— 59 —
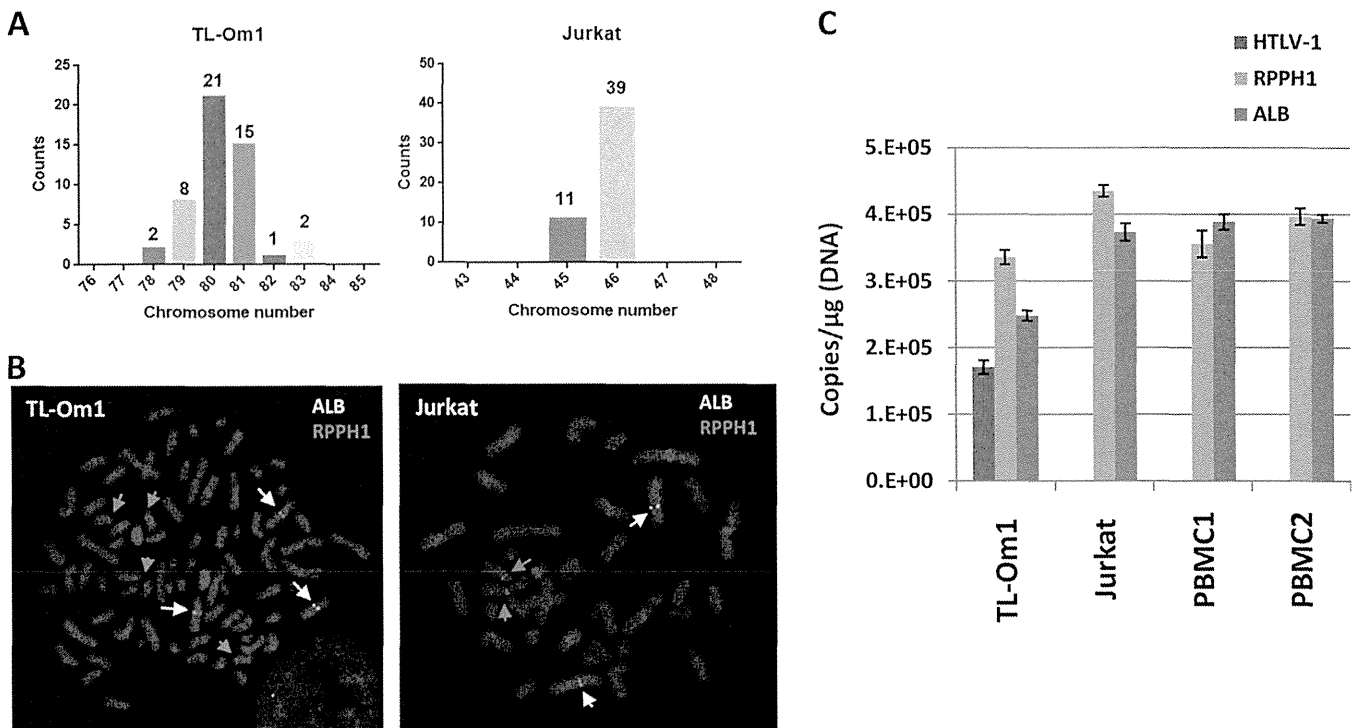
FIG 3 Gene copy number of IC cellular genes for HTLV-1 qPCR. (A) The number of chromosomes in TL-Om1 and Jurkat cells at metaphase was counted. Horizontal line indicates the number of chromosomes per cell. (B) Representative FISH images of TL-Om1 and Jurkat cells at metaphase. Yellow and red arrows indicate signals for ALB and RPPH1 probes, respectively. Left panel shows three signals for ALB and four for RPPH1; right panel shows two signals for ALB and two for RPPH1. (C) Determination of the gene copy number of HTLV-1, RPPH1, and ALB genes by digital PCR. gDNA of TL-Om1 and Jurkat cells and of PBMCs from two healthy donors were subjected to digital PCR. Data show the absolute copy number of HTLV-1, RPPH1, and ALB genes per microgram of gDNA. Bars are means from triplicate analyses.

In vitro HTLV-1 infectivity test. Frozen cells were thawed and immediately cultured for a week. Exponentially growing cells were used for the assay. Jurkat, TL-Om1, SLB1, and HUT102 cells were treated with 50 μg/ml mitomycin C (Kyowa Hakko Kirin, Tokyo, Japan) and incubated for 1 h at 37°C. After being washed twice with 2% FBS-PBS, 1×10^5 cells were added to culture medium containing 1×10^6 Jurkat cells. Mitomycin C was used to block the growth of ATL cell lines added to Jurkat cells. Cells were cocultured for 2 weeks and then subjected to qPCR to determine PVL, as described previously (11).

RESULTS

HTLV-1 infectivity in TL-Om1 cells. We investigated the production potential of infective virus to ascertain the clonal stability of HTLV-1 integration *in vitro*. Mitomycin C-treated TL-Om1 cells were cocultured with Jurkat cells for 2 weeks. At the end of the 2 weeks, no HTLV-1 integration was observed in the Jurkat cells that were cocultured with TL-Om1 cells, while HTLV-1 integration was observed when Jurkat cells were similarly cocultured with SLB-1 and HUT102 cells (Fig. 1A). These findings suggested that the production of infective HTLV-1 particles from TL-Om1 cells was low or diminished; thus, the increase in copy number over the course of cell culture was thought to be negligible. If TL-Om1 cells had infectious potential, the clonality of HTLV-1 provirus in them would vary because of the mutual HTLV-1 infections between cells. To evaluate the clonality of HTLV-1 provirus in TL-Om1 cells, TL-Om1 gDNA was analyzed by Southern blotting. EcoRI-digested gDNA showed a single band, while PstI digestion produced five DNA bands that contained an HTLV-1 sequence

(Fig. 1B). Three of the five DNA bands were HTVL-1 internal sequences. The other two DNA bands contained either 5' or 3' HTLV-1 sequences ligated with the host genome (Fig. 1B). These fragment patterns indicated that HTLV-1 provirus integration in TL-Om1 cells was monoclonal.

Determination of copy number and integration site of HTLV-1 provirus by FISH. To confirm the clonality and copy number of HTLV-1 provirus and of IC genes in detail, we performed a FISH analysis. There were one or two signals of HTLV-1 provirus in the cells. The mean proviral copy number was calculated at 1.8 copies/cell from the count of signals with >250 cells in two independent analyses (Fig. 2Ai and ii). Double-staining of the TL-Om1 genome with both HTLV-1 and 1q44 probes in meta-

TABLE 2 Gene copy number of IC genes determined by FISH

	Gene copy no.			
	TL-Om1 (20 analyzed cells)		Jurkat (20 analyzed cells)	
Karyotype	RPPH1 gene	ALB gene	RPPH1 gene	ALB gene
2N	0	0	20	20
3N	1	20	0	0
4N	19	0	0	0
Average	3.95	3	2	2
Ratio to the RPPH1 gene	1	0.76	1	1

— 60 —

TABLE 3 Summary of ratio of gene copy numbers to the RPPH1 gene

Method	Cell line	Gene copy no. ratio to the RPPH1 gene							
		RPPH1 gene	ALB gene	ACTB gene	CD81 gene	HBB gene	RAG-1 gene	HTLV-1 gene	LTR gene
FISH	TL-Om1	1.00	0.76					0.46	
	Jurkat	1.00	1.00						
Digital PCR	TL-Om1	1.00	0.74					0.51	
	Jurkat	1.00	0.86						
	PBMC1	1.00	1.09						
	PBMC2	1.00	0.99						
qPCR (plasmid)	TL-Om1	1.00	0.74					0.48	1.02
	Jurkat	1.00	0.92						
qPCR (gDNA)	TL-Om1	1.00	0.74	1.18	0.99	0.92	0.94		
	Jurkat	1.00	0.95	1.07	0.99	0.90	1.08		
	PBMC 1	1.00	0.99	1.00	0.98	0.99	1.00		
	PBMC 2	1.00	1.01	1.01	0.99	1.00	1.01		

phase showed that all HTLV-1 DNA signals were located on chromosome 1 (Fig. 2Bi). When the number of copies of chromosome 1 was 1, 2, 3, or 4 per cell, the number of HTLV-1 proviruses per cell was 1, 1, 2, and 2, respectively (data not shown). HTLV-1 signals on chromosome 1 were positioned on the band of 1p13 (Fig. 2Bii). These results correlated well with the Southern blotting results that showed monoclonal integration.

Confirmation of integration site of HTLV-1 in TL-Om1 cells. To identify the integration site of monoclonal HTLV-1 provirus, Splinkerette PCR was performed with TL-Om1 gDNA. Two specific PCR products were obtained by gel electrophoresis (Fig. 2Ci). The DNA fragments were analyzed by direct sequencing. Sequencing analysis of the lower-molecular-weight DNA fragments (Fig. 2Ci, lower band) showed that they were provirus genomic sequences. Sequencing analysis of the higher-molecular-weight band showed that it contained host gDNA ligated to the 3′ LTR of HTLV-1. We also performed inverse PCR with TL-Om1 gDNA that was digested with BamHI or XbaI followed by self-ligation. Single DNA bands were obtained from both BamHI and XbaI self-ligated templates (Fig. 2Cii). Sequencing analysis demonstrated that both bands contained the same sequences. A BLAST search revealed that the sequence was located on chromosome 1. The integration site was identified, and the HTLV-1 provirus was integrated inversely in between the CATATAT repetitive sequences at the region of NT_077389 from nucleotides (nt) 164570 to 164576 on chromosome 1 (Fig. 2Di and ii).

We determined the full-length sequence of HTLV-1 provirus in TL-Om1 cells by genomic long PCR followed by direct sequencing. The length of HTLV-1 provirus was determined to be 8,941 bp (GenBank accession no. AB979451; see also Text S1 in the supplemental material). The percent identity to the HTLV-1 genomic sequence of the ATK-1 strain (accession no. J02029) was 98.7%. Compared with the full-length HTLV-1 genomic sequence of ATK-1, there was a 93-nt deletion in the *env* gene. The region that was deleted was equivalent to nt 5547 to 5669 of ATK-1. The deduced amino acid sequence of the deletion was 31 in-frame amino acids (Δ125–155 of Env). The deleted region was located on the receptor binding domain of Env (see Fig. S1 in the supplemental material).

Calculation of chromosome and gene copy numbers of HTLV-1, RPPH1, and ALB genes in TL-Om1 and Jurkat cells. We counted the chromosome number in TL-Om1 and Jurkat cells by FISH analysis. Jurkat cells were analyzed as one of the control cell lines. The chromosome number differed from 78 to 83 in TL-Om1 cells (Fig. 3A). The mean chromosome number was estimated at 80.2, which indicated that the karyotype of TL-Om1 cells was about 4N. There were 45 or 46 chromosomes in Jurkat cells, indicating that their karyotype is near that of normal human diploid cells (Fig. 3A and B and Table 2).

The absolute gene copy number of HTLV-1 provirus and IC genes was measured using digital PCR. gDNA from TL-Om1 cells, Jurkat cells, and PBMCs from two healthy donors was subjected to digital PCR and used to calculate the copy numbers of these genes (Fig. 3C). Although the ALB-to-RPPH1 gene copy number ratios in the two PBMC samples were 1.09 and 0.99, the ALB-to-RPPH1 gene copy number ratio in TL-Om1 cells was low (ratio of 0.74) (Table 3). The provirus-to-RPPH1 gene copy number ratio in TL-Om1 cells was 0.51 (Table 3). These results were consistent with the provirus- and ALB-to-RPPH1 gene copy number ratios estimated by FISH, which were 0.46 and 0.76, respectively (Table 3). The usefulness of TL-Om1 as a reference standard is strongly supported by the consistent results from the FISH and digital PCR analyses (Table 4).

Estimation of the gene copy number of HTLV-1 and IC genes by synchronized qPCR. We previously developed a method to determine inherited allelic deletions by using qPCR with primer sets that can amplify fragments synchronously, even though the

TABLE 4 Absolute gene copy number per microgram gDNA determined by digital PCR

| Cell line	Gene copy no./μg gDNA[a]		
	HTLV-1 gene	RPPH1 gene	ALB gene
TL-Om1	170,171.1	335,452.3	248,410.8
Jurkat	NT	434,529.6	373,423.9
PBMC1	NT	355,116.1	388,650.0
PBMC2	NT	397,260.3	394,520.5

[a] Data are means of triplicate analysis. NT, not tested.

— 61 —
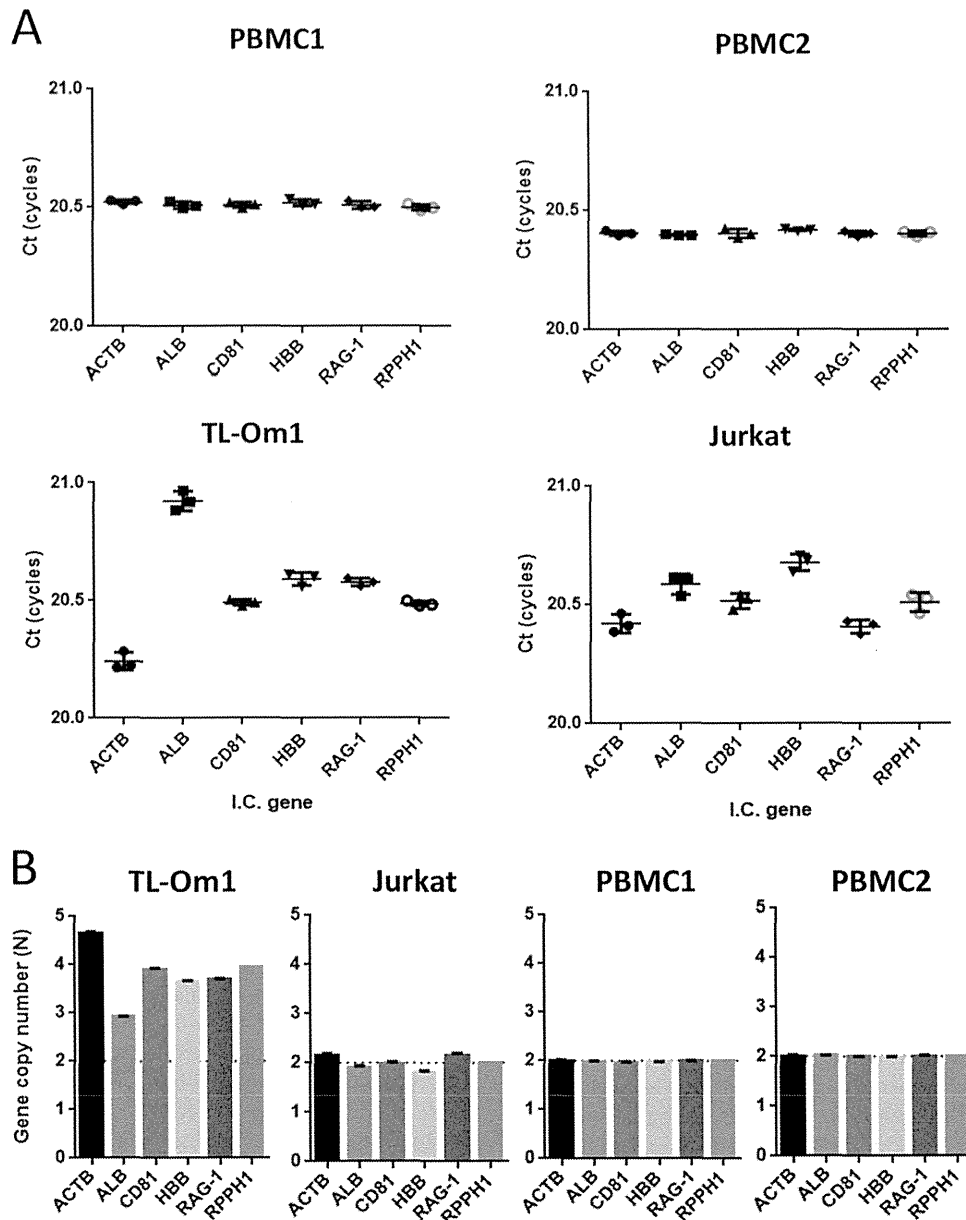
FIG 4 Estimation of gene copy number of IC genes in TL-Om1 cells by qPCR. gDNA of TL-Om1 and Jurkat cells and of PBMCs from two healthy donors was tested by qPCR with synchronous amplification primer sets for IC genes. (A) C_T scores (cycles) of each primer set for IC genes. Each dot indicates the mean from triplicate analyses. The C_T scores in the graph were the results of correction by the factors described in Table 1. (B) Estimated gene copy number of IC genes calculated using the difference in C_T scores from RPPH1. The copy numbers of IC genes of TL-Om1 and Jurkat cells were calculated based on FISH analysis for the RPPH1 gene. RPPH1 gene copy number from PBMCs was set as 2N. Equation for the estimation of gene copy number was as follows: gene copy number (N) = RPPH1 gene copy number determined by FISH analysis × $2^{-\Delta C_T}$, $\Delta C_T = C_T$(target gene) − C_T(RPPH1).

target genes are different. The method shows that the difference in C_T value determines the difference in gene copy number. We used primer sets for HTLV-1 genes (LTR and coding regions) and ACTB, ALB, CD81, HBB, and RAG-1 IC genes (Table 1). To increase the specificity, we used primer correction factors, which compensate for the slight difference in PCR amplification efficiency between different primers for target genes. As shown in Fig. 4A, TL-Om1 and Jurkat cells did not show the complete synchronized amplifications that were observed in normal PBMCs. By setting the PCR amplification efficiency of all primer sets per cycle

to approximately 2-fold, the ratio of the gene copy number against the RPPH1 gene was estimated using the difference in the mean C_T scores of the IC gene primer sets from the mean of those for the RPPH1 gene. The ratios of the gene copy number of the ALB gene to that of the RPPH1 gene in TL-Om1 and Jurkat cells were 0.74 and 0.92, respectively (Table 3). When the copy number of the RPPH1 gene in TL-Om1 cells was set at 3.95, which was determined by FISH analysis, the copy number of the IC genes was at least 2.9 (ALB gene) and at most 4.7 (ACTB gene) (Fig. 4B and Table 3).

— 62 —
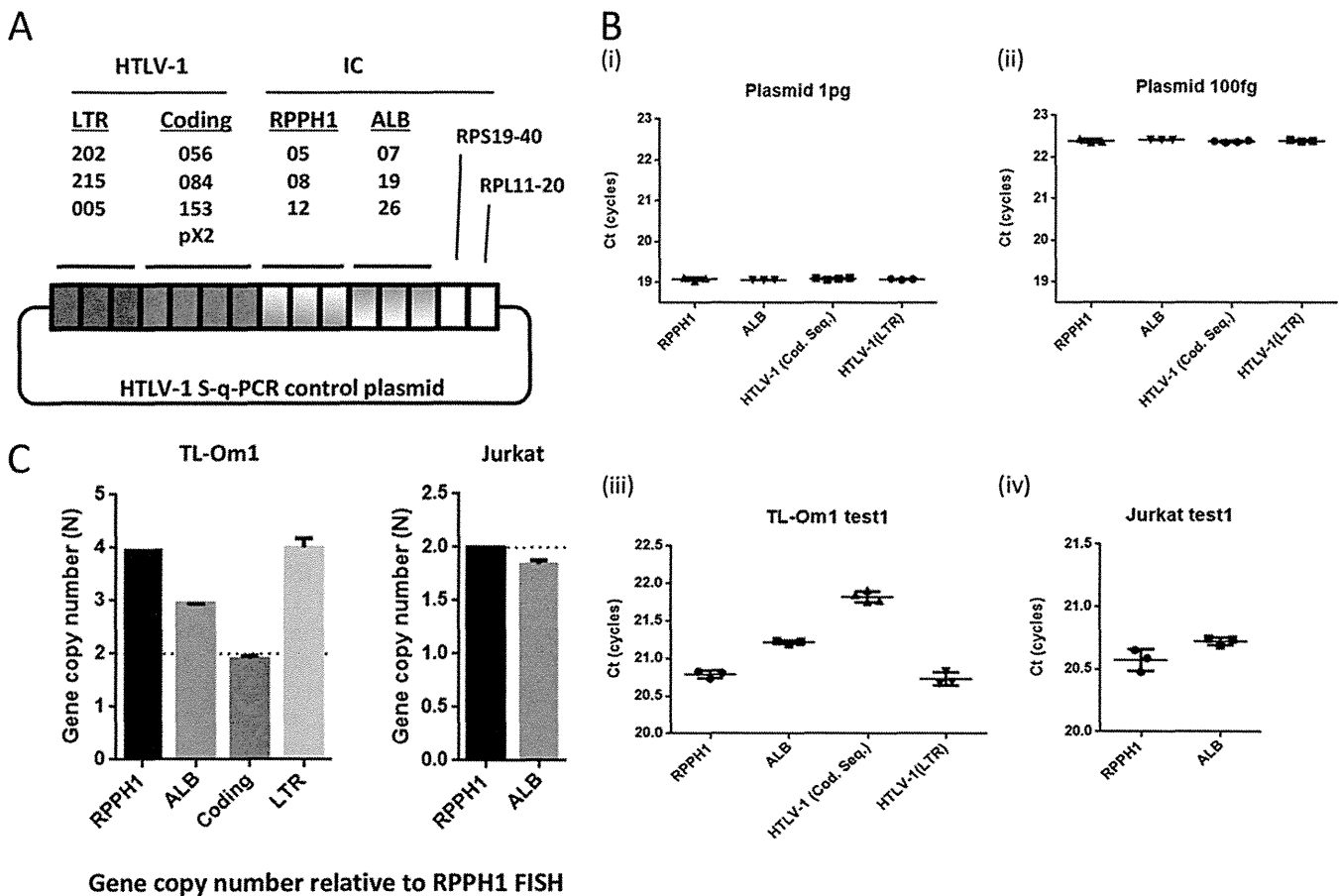
FIG 5 Estimation of the HTLV-1 gene copy number in TL-Om1 cells by synchronized qPCR. gDNA of TL-Om1 and Jurkat cells and of PBMCs from two healthy donors was tested for qPCR with synchronous amplification primer sets for HTLV-1, RPPH1, and ALB genes. (A) Construction of control plasmid with a single copy of each target sequence; (B) data indicate C_T scores of HTLV-1, RPPH1, and ALB genes for control plasmid at 1 pg, 100 fg/reaction, and for TL-Om1 and Jurkat cells. qPCR with the plasmid showed synchronous amplification of all primer sets. Each dot indicates the mean from triplicate analyses. The C_T scores in the graph are the results of correction by the factors described in Table 1. (C) Estimated HTLV-1 and ALB gene copy number in TL-Om1 and Jurkat cells. Data were estimated using the difference in C_T scores between target genes and RPPH1 genes.

Additionally, we tried to determine the HTLV-1 copy number in TL-Om1 cells using a synchronized qPCR method. We prepared a plasmid that had one copy of every target PCR amplicon (Fig. 5A). The plasmid had the same copy number as all the target regions. Using the plasmid as a template, we performed qPCR and confirmed the synchronized amplification of primer sets for HTLV-1, RPPH1, and ALB genes (Fig. 5B). The difference in mean C_T scores for the HTLV-1 gene to the RPPH1 gene was 1.05 cycles on average in TL-Om1 cells (Fig. 5C and Table 3). As with the sequencing analysis, use of the synchronized qPCR method also estimated the copy number of the LTR to be 4.01, indicating that TL-Om1 cells have two LTRs (Fig. 5C and Table 3).

Comparison of HTLV-1 copy number from different calculation methods. We compared the results of HTLV-1 and ALB gene copy number obtained from FISH, digital PCR, and synchronized qPCR. The copy number ratios of the HTLV-1 gene to the RPPH1 gene in TL-Om1 cells were 0.46, 0.51, and 0.48, from FISH, digital PCR, and synchronized qPCR, respectively, and those for the ALB gene were 0.76, 0.74, and 0.74 (Fig. 6 and Table 3). The results from these varied assays strongly support one another, indicating that TL-Om1 cells are suitable for use as a reference material for HTLV-1 qPCR.

DISCUSSION

Recently, NAT reference materials have been established for the safety of blood and blood products, such as international standards for HIV, hepatitis B virus, and hepatitis C virus (22–24). These materials have been frequently used for the purpose of calibration and validation of test systems, preparation of secondary reference materials, and comparison of multicenter results, which have helped improve the consistency of the results. Most international standards for blood-transmitted viruses use plasma from infected human blood, because the test target is extracted from human plasma. With regard to HTLV-1 NAT, it may be better to use a cell line as a reference material to standardize the qPCR results, because this test uses cells obtained from peripheral blood. An example of NAT reference material using cell lines is reported in a test for quantitation of BCR-ABL mRNA. Panels of K562 cells combined with HL60 cells were set as standards, which have been approved by the WHO Expert Committee of Biological Standardization (25). Although a variety of cell lines harboring HTLV-1 provirus in their genomes has been established, detailed characterization of the candidate cell lines with regard to their suitability as reference materials for HTLV-1 NATs has not yet been per-

— 63 —

A

TL-Om1

Jurkat
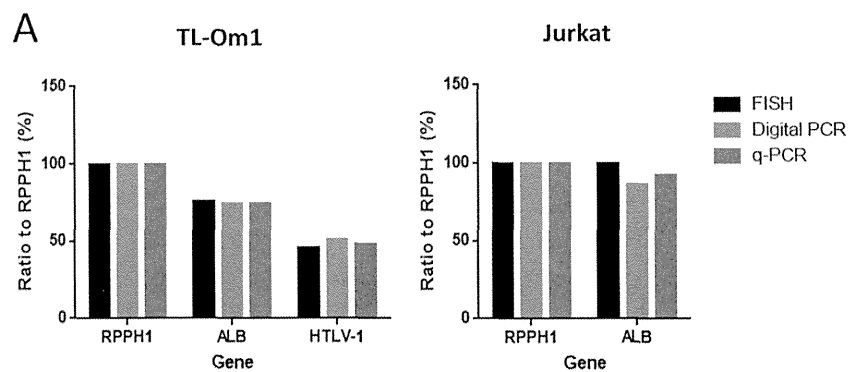
FIG 6 HTLV-1- and ALB-to-RPPH1 gene copy number ratios. (A) Comparison of the HTLV-1- and ALB-to-RPPH1 gene copy number ratios determined by FISH, digital PCR, and qPCR. Data indicate percentages of gene copy number ratio to the RPPH1 gene.

formed. Among the HTLV-1 cell lines, TL-Om1 is well known to be latently infected with HTLV-1 and is thought to be stable for HTLV-1 clonality (17, 26). Transcription from HTLV-1 provirus in TL-Om1 cells is blocked by the highly methylated LTR (27).

In this study, we evaluated the distinct genomic properties of HTLV-1 and IC genes in TL-Om1 cells with regard to their suitability as reference materials for HTLV-1 NATs. Precise information about HTLV-1 infectivity, karyotype, and absolute copy number of HTLV-1 and cellular control genes of TL-Om1 is useful for applying TL-Om1 as a reference material for HTLV-1 qPCR. As such, for this use, TL-Om1 has advantages over other cell lines, such as the human ATL cell line MT2 and the rat T-cell line TARL-2. A recent study of HTLV-1 testing in Japanese blood donor screening revealed that virus prevalence is not limited to areas where HTLV-1 is endemic but has shifted to the entire country, especially the Tokyo metropolitan area (6). Nationwide HTLV-1 tests have been performed on pregnant women in Japan since the end of 2010. The frequent occurrence of seroindeterminate results after Western blotting is one weakness of the HTLV-1 antibody tests. HTLV-1 qPCR is thought to be a solution for decreasing the number of seroindeterminate results; therefore, an accurate measurement of HTLV-1 proviral DNA by qPCR is needed. Additionally, a PVL value of >4% in PBMCs is reported to be a risk factor for ATL development from HTLV-1 asymptomatic carriers, which emphasizes the importance of measuring PVL by qPCR (7). PVL monitoring also provides a risk indicator for HAM/TSP (8).

An attempt to minimize the differences between laboratories by using a common plasmid that included the pX region has been reported. When standard curves were constructed by utilizing the common plasmid in all participating laboratories, the differences in median intralaboratory coefficient of variation (CV) could be reduced by about half (16). Although the attempt worked well among participating laboratories with in-house qPCR methods, the transferability of utilizing common plasmids for standard curves to other methods for PVL determination, for example, digital PCR, is uncertain.

To standardize HTLV-1 qPCR, we advocate the use of TL-Om1 cells with finely elucidated HTLV-1 genomic information as reference material. A previous report showed that PVL values of males and females, on average, are 1.39% and 2.10%, respectively (7). Thus, a dilution or a serial dilution of TL-Om1 with PBMCs or Jurkat cells at a PVL value of around 2% would be an appro-

priate material for the standardization of HTLV-1 qPCR. These kinds of references can be easily prepared, because the absolute gene copy number is determined from the dilution rate of TL-Om1. TL-Om1 cells were also used as a control in a deep-sequencing-based method for the quantification of the clone size of HTLV-1-infected cells in HTLV-1 carrier or ATL patients (28).

We conclude that TL-Om1 cells can be used as a useful reference material for HTLV-1 NATs. By using TL-Om1 cells, researchers will be able to define the exact values of HTLV-1 by quantifying the copy numbers of provirus and IC genes. In the future, we hope that other laboratories will utilize the features of TL-Om1 cells to standardize the HTLV-1 qPCR.

ACKNOWLEDGMENTS

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— 65 —

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輸血用血液における病原体不活化技術の現状と
新規技術の開発

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┃ はじめに

　輸血用血液は，問診に加えて，病原体の血清学検査法とウイルス遺伝子を高感度に検出できる核酸増幅法の導入によって感染症の発生頻度は急激に低下したものの，スクリーニング法の限界や検査が実施されていない病原体などの感染リスクが存在する．そのため，輸血の安全性を確保するための対策として，病原体の不活化法が検討されるようになった．現在のところ，血漿製剤と血小板製剤の病原体不活化法が実用

図1 病原体の不活化法の評価

化され，欧州の一部の国や地域で導入されている．しかし，輸血用血液で最も使用量が多い赤血球製剤では，実用化された方法はない．

　本稿では，輸血用血液の不活化法の現状と問題点について述べる．

┃ 病原体不活化法の
┃ 評価法と効果について

　不活化法を実施すれば，混入している病原体が全て不活化（感染力を失うこと）できるわけではない．不活化法にはそれぞれ不活化できる限界が存在する．不活化の能力を超えた量の病原体が混入していれば，感染価は減少するものの感染性を有した病原体が存在することになり，受血者の感染につながる．

　日本では「不活化」という表現を使用するが，欧米では「低減化：リダクション」という言葉もよく使用されている．不活化効率は，**図1**に示すように，血液バッグに評価用の病原体を添加し，血液バッグに含まれる感染性を有する総病原体量（T1×V1）を計算する．次に不活化処理後の総病原体量（T2×V2）を計算し，リダクション値 $R = \log(V1 \times T1/V2 \times T2)$ として求められる[1]．

　ウイルスの場合，少量でも感染性ウイルスが
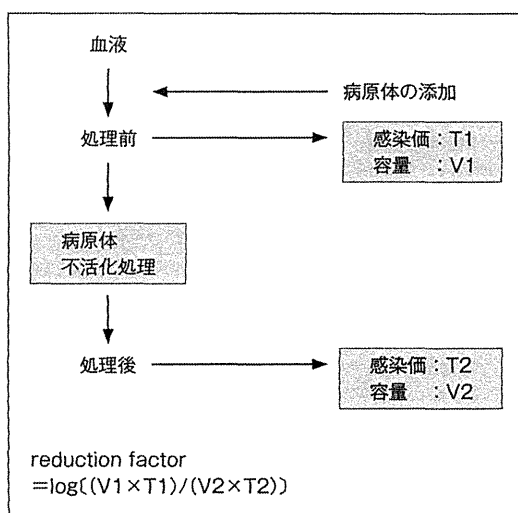
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表 1 血漿および血小板製剤の不活化法

方法	S/D 処理法	メチレンブルー法	アモトサレン法	リボフラビン法	(参考)検疫
対象製剤	血漿	血漿	血漿 血小板	血漿 血小板	血漿
製剤を構成する供血者数	多数	単	単	単	単
添加薬剤	界面活性剤	メチレンブルー	アモトサレン	リボフラビン	不要
照射	不要	可視光	紫外線A 320〜400 nm	紫外線B 265〜370 nm	不要
添加薬剤の除去	+	+	+	不要	不要
使用実績	◎	◎	○	△	◎

残存していれば感染する可能性があるので，血液中のウイルス量が少ないウイルスに対しては効果的であるが，ウイルス量が多い感染症に対しては，処理能力の範囲までスクリーニング法などを併用して混入するウイルス量を減らさないと，不活化法単独で完全に病原体の感染を防ぐことは困難である．実際に不活化法を導入している血液センターにおいては，HBV(hepatitis B virus)，HCV(hepatitis C virus)，HIV(human immunodeficiency virus)の3つの血清学的に加えて核酸増幅法も併用している．不活化法の導入でこれら3つのウイルスのスクリーニング検査を止めた施設はない．

以上の記載だけでは，不活化法単独では利用価値がない技術と誤解されるかもしれないが，血小板の細菌感染予防では大いに効果を発揮している．これについては，「血小板の病原体不活化法」の項で後述する．

血漿の病原体不活化法

現在，実用化されている血漿の病原体不活化法には，S/D(solvent/detergent)処理法，メチレンブルー法，アモトサレン法，リボフラビン法の4つがある[2]．

S/D 処理法は欧州で主に導入され，数十 L〜数百 L の血漿を混ぜ合わせてから，界面活性剤と変性剤を加えて病原体を処理する方法である(一般的に S/D プラズマと呼ばれている)．S/D 処理法は，エンベロープを有するウイルス(HIV・HBV・HCV など)に対しては極めて有用な病原体不活化法である．その一方で，エンベロープをもたないウイルス(A 型肝炎ウイルス・E 型肝炎ウイルス・パルボウイルス B19 など)に対しては全く効果はない．

メチレンブルー法やアモトサレン法，リボフラビン法は，個々のバッグに化学物質を添加し，それぞれ可視光，紫外線 A，紫外線 B を照射することによって病原体を不活化する方法である．S/D 処理法と異なり，混ぜ合わせることなく個々のバッグで不活化処理できる大きなメリットがある．しかも，エンベロープを有するウイルスだけでなく，エンベロープをもたないウイルスに対しても不活化することができる(ウイルスによって，抵抗性を有していて全く不活化できないものや，不活化効率が低いものもある)[3]．問題点には，添加する化学物質の毒性に懸念があることや，凝固因子などの活性がある程度失活し，低下することであることが挙げられる[2]．各不活化法の特徴を表1に示す．

参考までに，不活化法のほかに検疫(quarantine)を紹介する．これは，製剤を一定期間保管(わが国では6カ月)し，供血者が次回の献血時の検査や問診で問題がないことが確認された場合や，同じ血液から製造された赤血球製剤を投与された受血者から異常の報告がなかった場合に，医療機関に凍結保存されていた血漿を供給する方法である．採血から供給まで長時間を要するが，不活化処理のための化学物質の毒性を考慮する必要はない．

血小板の病原体不活化法

現在，実用化されている方法にはアモトサレン法とリボフラビン法がある(表1)．方法は血漿と同様である．血小板は室温(20～24℃)で震とうしながら保存されるため，その間に細菌が増殖し，致死的な量まで増殖する可能性がある．細菌の混入する原因として，採血時に毛穴に存在する細菌が皮膚の断片と一緒に血液バックに入ることや無症候性の菌血症(健常人であっても口腔内などの菌が血液中に入ることがある)が挙げられる．対策として，最初の血液30 mLを検査用の検体として別のバックに取り，その後の血液を製剤用バックに取る(初流血除去)方法や細菌培養法が実施されている．しかし，初流血除去では，混入頻度は減らせても完全に予防はできない．さらに，無症候性の菌血症には無効である．

一方，細菌培養法では，混入する細菌数が少ないため〔1バッグ当たり10～100 CFU(colony-forming unit)〕，偽陰性となることがある．さらに，試験結果が出る前に使用せざるを得ないなど，無菌検査を導入しても細菌の混入を完全には検出することはできない．欧米では，採血日を入れないで有効期間が5～7日間と長い．

さらに，5人前後の供血者の全血からの血小板を1つに集めて血小板製剤を製造しているため，細菌の混入する率が高くなる．一方，日本では採血日を入れて有効期間は4日なので，血小板への細菌感染は欧米に比べて少なく，初流血除去の導入後死亡例は報告されていない．

病原体の不活化が血小板の機能に与える影響は，臨床的に血小板輸血後の血小板増加数(corrected count increment, CCI：1時間後の補正血小板増加数$CCI_{1時間}$と24時間後の$CCI_{24時間}$)，血小板輸血回数，赤血球輸血回数・間隔，出血症状などから評価される．血小板の投与は，臨床的には予防的な投与もあることから不活化による機能低下の評価は難しいが，無処理と差はないという報告が多い．また，不活化された血小板輸血によって細菌感染が生じたという報告もない．

赤血球製剤の病原体不活化法

赤血球は輸血のなかで特に使用頻度・量とも多い製剤であるが，実用化された不活化法はない．血漿や血小板に用いられている方法は，化学物質に可視光，または紫外線を照射して病原体の核酸を破壊する方法であるため，赤血球製剤では，赤血球に照射された光が吸収されてしまい，病原体を不活化できる十分な光が病原体に到達しないためである．

近年，光を必要としない新しい不活化法が開発され，本年度フェイズⅢの治験が予定されるところまでになった[4]．この方法は，S-303と呼ばれるquinacrine mustard類似のアルキル化剤を使用し，アンカー，リンカー，エフェクターの3つの部分から構成されている．アンカーで核酸内に挿入されエフェクターが核酸に結合し核酸の複製を阻害する．添加された薬剤

はリンカー部分で加水分解される．ヘマトクリット60％でも幅広い病原体を不活化することが可能であるといわれている．この不活化法が実用化されると，3つの輸血用製剤にそれぞれ不活化法が実用化されたことになる．

▍おわりに

1. 輸血を介する感染症の頻度は急激に低下したが，リスクは0ではない．
2. 病原体（ウイルス）の不活化法には不活化可能な限界があるため，不活化法の導入によってウイルスのスクリーニング検査が全て廃止できるわけではない．
3. 病原体の不活化法によって血漿や血小板の機能は低下する．臨床的に容認できる範囲であることが必要である．
4. 血小板の細菌感染が輸血後感染症の大きな

リスクとなっている国（地域）では，細菌感染予防のために不活化法は有用である．

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