primary effusion lymphoma : PEL

腫瘍性疾患―非Hodgkinリンパ腫・B細胞性

原発性滲出液リンパ腫

疾患の概要

- PELは，胸水や腹水などの体腔液中に浮遊する形で増殖するB細胞性の液性リンパ腫であり，通常，腫瘍塊を形成しない．
- ヒトヘルペスウイルス8（human herpesvirus 8：HHV-8またはKaposi's sarcoma associated herpesvirus：KSHV）が腫瘍細胞核内に検出される．WHO分類の定義ではHHV-8陰性の症例はPELに含めない．したがって，**HHV-8の検出がPELの診断には必須である．**
- AIDSなどの免疫不全に関連して発症する例がほとんどを占める．
- 胸膜などの隣接する部位に固形腫瘍を形成することがある．また，まれに液性リンパ腫の形をとらず，HHV-8関連固形リンパ腫として現れることがあり，これはextracavity PEL（体腔外PEL）と呼ばれている．

染色体・遺伝子異常

- 報告されている染色体・遺伝子異常はない．

病態発生機構

- HHV-8による日和見腫瘍であり，すべてのリンパ腫細胞からHHV-8が検出される．HHV-8はEBVと同じγヘルペスウイルスに属し，Bリンパ球に潜伏感染するウイルスである 図1 ．

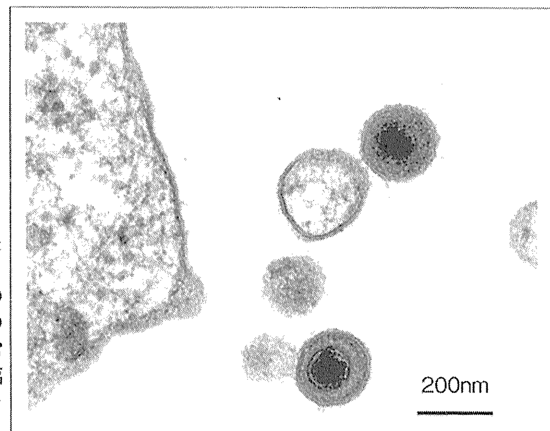


図1 HHV-8のウイルス粒子
フォルボルエステルで刺激したHHV-8持続感染細胞株BCBL-1に見られたウイルス粒子．他のHHVと近似し，約200nm程度の直径をもつ．なお，HHV-8は通常潜伏感染しているため，PELの臨床検体から電顕でウイルス粒子を見ることはできない．

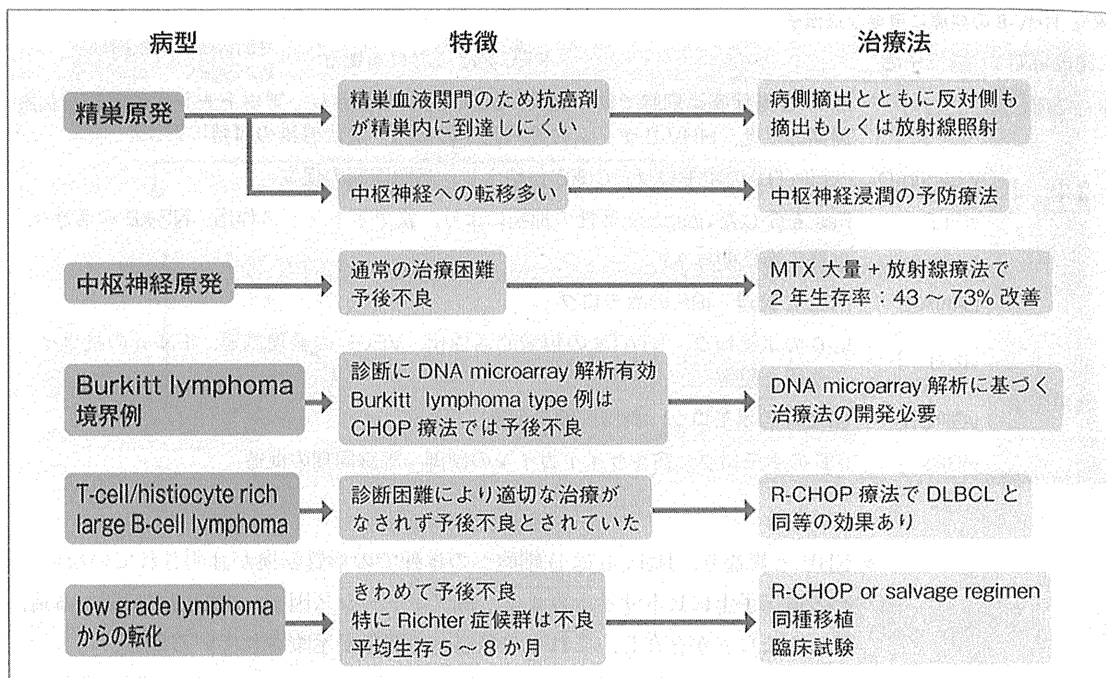


図13 DLBCL の特殊病型と治療法

トポシド，シタラビン，ブレオマイシン，ビンクリスチン）療法のランダム化比較試験において生存率に関して 4 者間に有意差がないことが 1993 年に報告され，以降，CHOP 療法が aggressive lymphoma の標準的治療法となった．1990 年代に B 細胞性リンパ腫に発現する CD20 に対するキメラ抗体薬剤リツキシマブが開発され，CHOP 療法とリツキシマブを併用する治療法が開発された．本治療法と CHOP 療法のランダム化比較試験にて DLBCL 例の生存率が 10〜20%改善することが報告され，以後 DLBCL 治療の標準治療はリツキシマブ併用 CHOP 療法となった．

（青笹克之，水木満佐央）

表1 HHV-8の発癌に重要な遺伝子

発現時期	遺伝子名	PEL発症における働き
潜伏感染	LANA-1	HHV-8潜伏感染細胞で常に発現．p53, Rb1と結合し，アポトーシスの阻害，細胞周期の促進．HHV-8ゲノムの娘細胞への伝搬．潜伏感染の維持
	v-cyclin-D	cyclin D1のホモログ．Cdk6と結合し，細胞周期の促進
	vFILP	Fasを介したcaspase活性の抑制により，抗アポトーシス作用．NF-kBの活性化
	kaposin	形質転換に関与？
	LANA-2	PELに発現．IRFのホモログ
増殖感染	vIL-6	IL-6のホモログ．STAT3の恒常的活性化．VEGFの発現誘導．IFN-αの抗ウイルス作用を阻害
	vGPCR	GPCRのホモログ．細胞周期の促進
	vIRFs	IRFのホモログ．宿主サイトカインの制御．細胞周期の促進

- EBVと異なり，HHV-8はB細胞への単独での形質転換が証明されていないが，その遺伝子上にヒトサイトカイン，抗アポトーシス因子，細胞周期に関わる遺伝子のホモログが存在し，これらの発癌への関与が示唆されている 表1 ．
- PELやKaposi肉腫ではHHV-8は潜伏感染状態にあるが，ごく一部の細胞では増殖感染状態に移行し，増殖感染関連蛋白を発現する．特にHHV-8の潜伏感染蛋白であるlatency associated nuclear antigen 1（LANA-1）の働きは重要で，p53やRb1と結合し，細胞周期を促進する働きと，HHV-8 DNAを染色体に結合させ，細胞分裂時に宿主DNAとともにHHV-8 DNAを複製し，娘細胞にHHV-8を伝搬し感染を持続させる働きをもつ．
- 遺伝子発現プロファイルの解析から，PELはpost germinal center B-cellが由来と考えられ，plasma cellのマーカーであるCD138が陽性である．なお，EBVも多くのPEL症例で検出されるが，陰性の症例が存在することや，培養によりEBVのみが脱落する症例があることからEBVの感染はPELの病変形成に必須でないと考えられている．

臨床所見

既往歴等，好発年齢，性

- 若年～成年のHIV陽性男性同性愛者がほとんどを占める．約半数にはKaposi肉腫の合併がみられる．
- まれながら移植後のレシピエント，高齢者での発症がある．

初発症状

- 胸腔，腹腔，心嚢腔が好発部位であり，こうした体腔液中に滲出液（胸水，腹水，心嚢水）が貯留する．リンパ節腫脹を伴わないのが普通である．
- AIDS合併例ではKaposi肉腫の既往がある症例が多い．
- extracavity PELは消化管，皮膚，肺などが多い．

血液・血清所見

- 通常，白血化はない．
- 血清中の HHV-8 DNA 量が上昇する例が多く，血清中には HHV-8 に対する抗体が検出される．
- 血中の IL-6 量が増加するが，ウイルスがコードする viral IL-6（vIL-6）の量も増加する．

病理所見

病理標本の作製上の注意

- PEL は腫瘍塊として増殖しないため，病理標本の作製には工夫が必要である．胸水などのサンプルではリンパ腫細胞の smear やサイトスピン標本の作製が必要である．
- 細胞数が多ければ遠心して沈澱とし，パラフィン標本の作製も可能である．また，胸膜などの隣接する部位に形成された腫瘍塊や extracavity PEL では，病変部の固形腫瘍から通常の病理標本を作製する．

細胞診標本

- リンパ腫細胞の smear やサイトスピン標本の Giemsa 染色では，リンパ腫細胞は大型の immunoblastic または plasmablastic な細胞から，anaplastic large cell 様の形態をとり，多彩である 図2 ．
- 核は多形性に富み，明確な核小体が特徴的で，細胞質は比較的広く，好塩基性であり，plasma cell にみられる核周明庭を思わせる所見もみる．

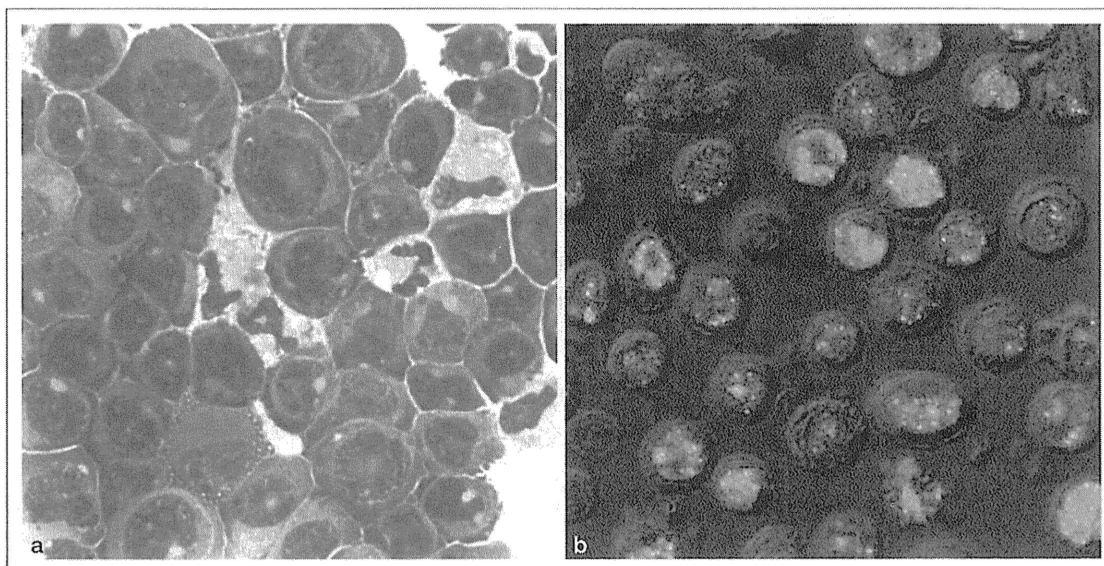


図2 PEL 症例の smear 標本
a：Giemsa 染色．大型の芽球様の細胞が見られる．核は多形性で，核小体が見られ，細胞質は広く，好塩基性に濃染する．核周明庭も見られ，plasma cell との類似を思わせる．
b：LANA-1 の蛍光免疫染色．ほとんどすべての細胞の核内に点状の陽性シグナルを認める．

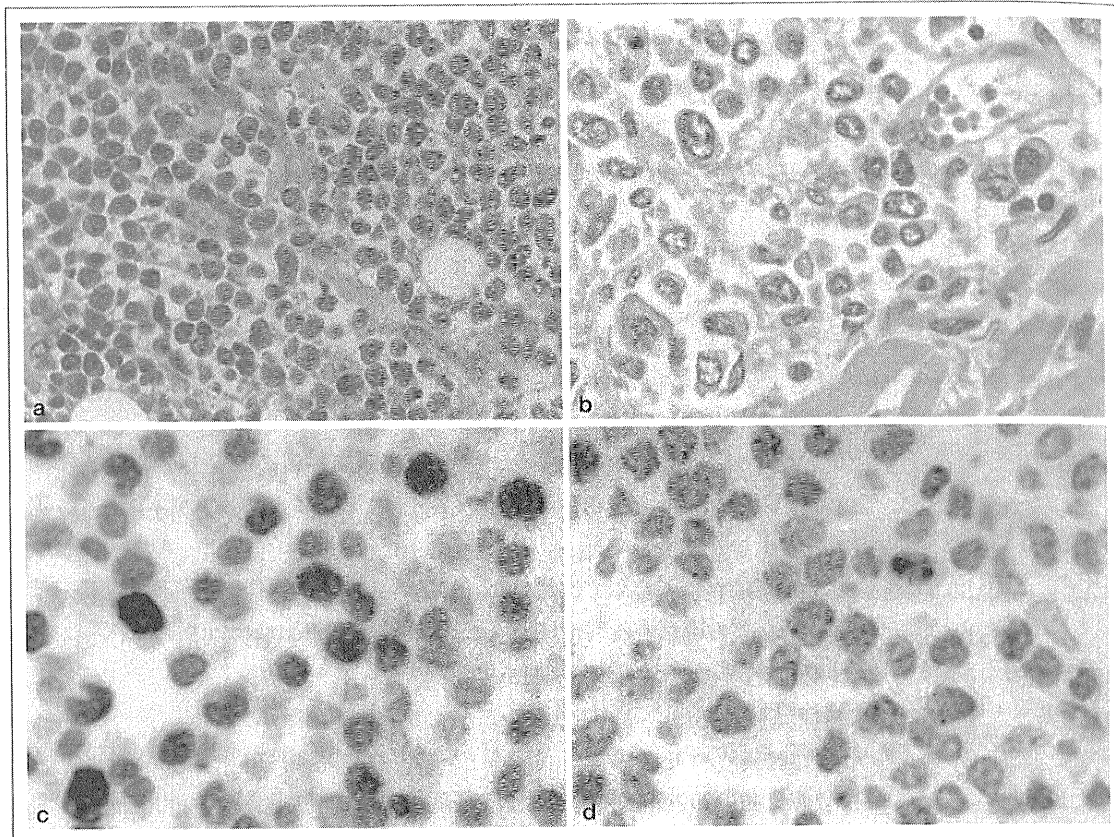


図3 extracavity PEL
a, b：HE 染色．大型の芽球様の細胞のびまん性浸潤を認める．核は偏在し，一部では車軸状である．細胞質は形質細胞に似る　c：EBV EBER の *in situ* hybridization　d：HHV-8 LANA-1 の免疫染色

組織標本

- extracavity PEL のパラフィン標本では大型で多形性に富む plasmablast ないし anaplastic large cell 様の細胞がびまん性に浸潤し，周囲との境界は不明瞭である 図3．
- 核は明瞭な核小体を有し，クロマチンが車軸状に見えるものもある．好塩基性の豊富な細胞質には核周明庭がみられ，plasma cell 様に見えるものもある．

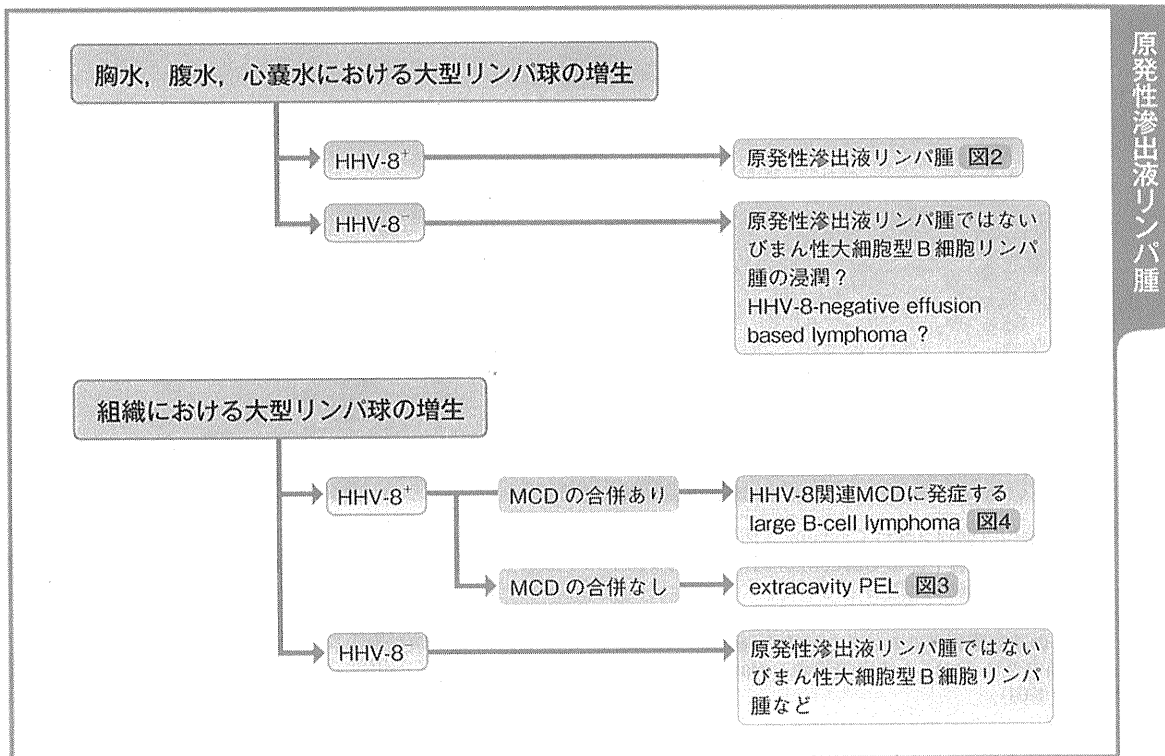
免疫組織化学，*in situ* hybridization

- CD45（LCA）$^+$であるが，CD20，CD79a などの B 細胞マーカーはすべて脱落している．
- CD30$^+$，CD138$^+$になることが多い．
- HHV-8 の潜伏感染蛋白である LANA-1 がリンパ腫細胞には必ず陽性であり，これが唯一の PEL のマーカーといえる 図3d．
- EBV は EBV-encoded small RNAs（EBER）が *in situ* hybridization で検出されることが多いが，EBER$^+$例でも latent membrane protein 1（LMP-1）$^-$であることが多い．
- extracavity PEL も同様の表面マーカーを発現する．

その他

- real time PCR を用いるとリンパ腫細胞に HHV-8 DNA が約 50 copy/cell で検出される．
- リンパ腫細胞の遺伝子検査ではイムノグロブリンの遺伝子再構成が認められる．

鑑別診断



▶ びまん性大細胞型 B 細胞リンパ腫(diffuse large B-cell lymphoma：DLBCL)

extracavity PEL は形態的に DLBCL と区別がつかない．HHV-8$^+$であれば DLBCL と同様の形態を示していても extracavity PEL とする．

⇒ extracavity PEL は plasmablastic または anaplastic large cell 様の形態をとること，B 細胞マーカーが欠如すること，CD30$^+$である症例が多いことなどが参考となる．

▶ HHV-8 陰性液性リンパ腫 (HHV-8-negative effusion based lymphoma)

C 型（または B 型）肝炎患者の腹水中に PEL と形態的によく似た液性リンパ腫が発症することが報告されている．

⇒これらは HHV-8$^-$であり，PEL とは異なる疾患である．

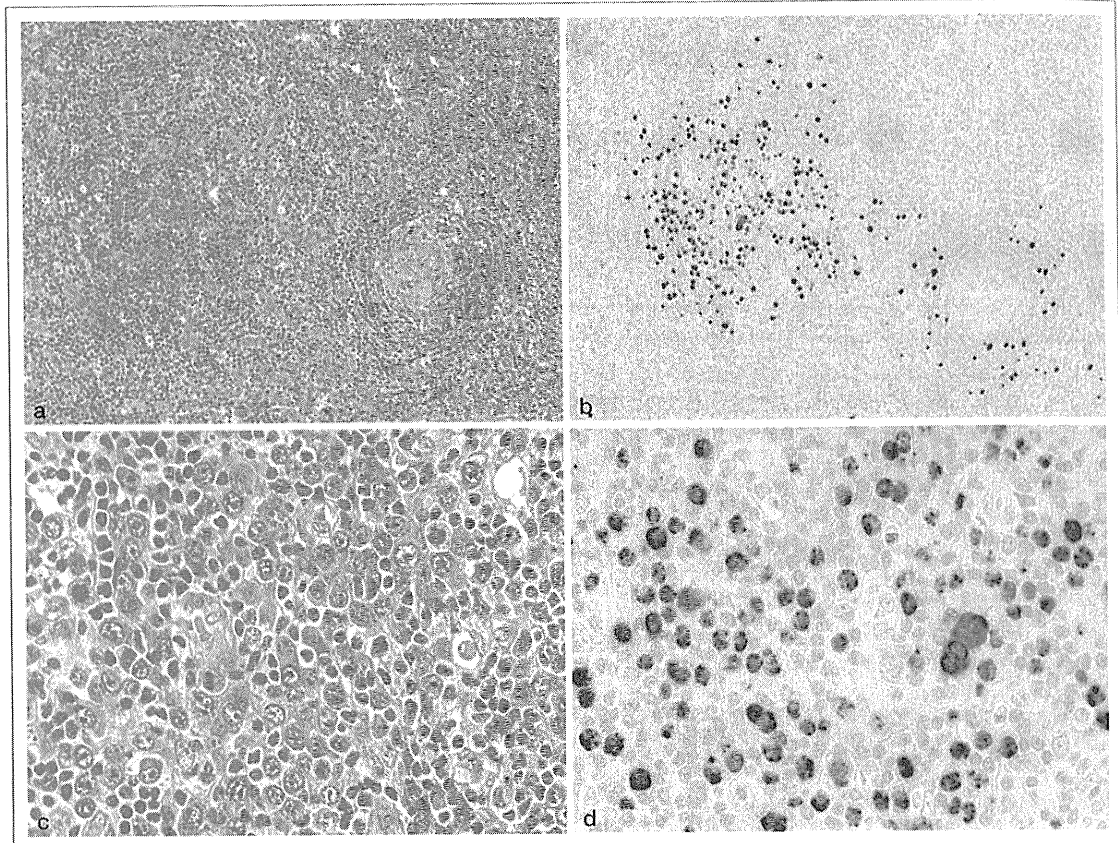


図4 HHV-8 関連多発性 Castleman 病に発症する大細胞性 B 細胞リンパ腫
a：HE 染色．右下には Castleman 病に典型的な硝子化した胚中心が見られ，暗殻は同心円状に拡大している．濾胞間には plasma cell の増生が見られる．
b：a の LANA-1 の免疫染色．リンパ濾胞暗殻と濾胞間組織に LANA-1^{+}細胞が見られる．
c：b の濾胞間組織における LANA-1^{+}細胞の HE 染色．plasma cell 様細胞が集簇している．
d：同部位の LANA-1 の免疫染色．plasma cell 様細胞に LANA-1^{+}で，この部位はいわゆる micro-lymphoma といえる（本文参照）．

▶膿胸関連リンパ腫（pyothorax-associated lymphoma：PAL）

PAL は結核性膿胸患者の胸膜に発症する EBV 関連の固形リンパ腫であり，HHV-8^{-}である．

⇒ PEL は胸水中に液性リンパ腫として発症する点でも区別できる．

診断のポイント

- 本邦では HIV 陽性男性同性愛者に発症がほぼ限定されることから，既往歴の確認が重要である．
- 胸水，腹水などからリンパ腫細胞が検出され，通常，腫瘍塊を形成しない．
- HHV-8^{+}であることは PEL 診断の必須条件である．
- リンパ節腫大または脾腫がある場合は HHV-8 関連 multicentric Castleman 病（MCD）に発症する large B-cell lymphoma を疑う．

▶ HHV-8関連多発性Castleman病に発症する大細胞性B細胞リンパ腫 (large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease) 図4

HHV-8関連の多発性Castleman病（MCD）から発症するリンパ腫であり，PELと同じ危険因子の患者（AIDS，男性同性愛者，Kaposi肉腫合併）に発症する．形態的にplasma cellに似ることからHHV-8-positive plasmablastic lymphomaともいわれる．

MCDを発生母地とすることから，リンパ節や脾臓に発症し，リンパ節腫脹や脾腫として現れる．組織学的にはCastleman病の病変部では胚中心の同心円状の硝子様物質の沈着と中心に向かって入り込む血管が特徴の，いわゆるonion skin appearanceを示し，濾胞間領域には多くのplasma cellの増生が認められる．HHV-8はマントル層および濾胞間のplasma cellの一部に陽性で，HHV-8^{+}細胞が小さな集塊を形成し，シート状に増生している像もみられる（micro-lymphomaと呼ばれる）．

免疫組織学的にはHHV-8 LANA-1^{+}で，HHV-8の溶解性感染関連蛋白であるvIL-6も多くの細胞で陽性である．

➡ cIgM^{+}，λ light chain^{+}である点はPELとは異なる．Castleman病の存在がPELとの鑑別には重要である．

治療

- 現在のところ，PELに対する確立した治療法はない．無治療での生存期間の中央値は2～3か月であり，一般に予後は悪い．
- CHOP療法などの化学療法に加え，highly active anti-retroviral therapy (HAART) を併用することで治療成績の改善がみられる．HAARTのみでPELが消退した症例報告もみられ，HAARTによる免疫の再構築がPELの治療にも有利に働くものと考えられる．
- PELはCD20^{-}であるためにリツキシマブは使用されない．

（片野晴隆，佐多徹太郎）

Journal of Medical Virology 83:322–330 (2011)

A Novel Real-Time PCR System for Simultaneous Detection of Human Viruses in Clinical Samples From Patients With Uncertain Diagnoses

Harutaka Katano,[1]* Motofumi Kano,[1] Tomoyuki Nakamura,[1] Takayuki Kanno,[1] Hideki Asanuma,[2] and Tetsutaro Sata[1]

[1]*Department of Pathology, National Institute of Infectious Diseases, Shinjuku, Tokyo, Japan*
[2]*Influenza Virus Research Center, National Institute of Infectious Diseases, Shinjuku, Tokyo, Japan*

A novel simultaneous detection system for human viruses was developed using a real-time polymerase chain reaction (PCR) system to identify causes of infection in clinical samples from patients with uncertain diagnoses. This system, designated as the "multivirus real-time PCR," has the potential to detect 163 human viruses (47 DNA viruses and 116 RNA viruses) in a 96-well plate simultaneously. The specificity and sensitivity of each probe–primer set were confirmed with cells or tissues infected with specific viruses. The multivirus real-time PCR system showed profiles of virus infection in 20 autopsies of acquired immunodeficiency syndrome patients, and detected frequently TT virus, cytomegalovirus, human herpesvirus 6, and Epstein–Barr virus in various organs; however, RNA viruses were detected rarely except for human immunodeficiency virus-1. Pathology samples from 40 patients with uncertain diagnoses were examined, including cases of encephalitis, hepatitis, and myocarditis. Herpes simplex virus 1, human herpesvirus 6, and parechovirus 3 were identified as causes of diseases in four cases of encephalitis, while no viruses were identified in other cases as causing disease. This multivirus real-time PCR system can be useful for detecting virus in specimens from patients with uncertain diagnoses. ***J. Med. Virol. 83:322–330, 2011.***
© 2010 Wiley-Liss, Inc.

KEY WORDS: real-time PCR; acquired immunodeficiency syndrome (AIDS); virus; autopsy

INTRODUCTION

Polymerase chain reaction (PCR) is a powerful tool to detect viruses compared with some traditional methods such as the direct fluorescent-antibody assay or virus isolation in cell culture. Real-time PCR is a sensitive system to detect viral genomes, used commonly worldwide [Storch, 2000]. Moreover, multiplex PCR fluorescence techniques are able to identify several genes in one tube simultaneously. Some reports have described simultaneous detection systems for up to 20 viruses using real-time PCR or conventional PCR [Vet et al., 1999; Bellau-Pujol et al., 2005; Li et al., 2007; Mahony et al., 2007; Molenkamp et al., 2007; Nolte et al., 2007; van de Pol et al., 2007; Wada et al., 2009]. However, the number of viruses detectable in one tube is limited by fluorescence wavelength. On the other hand, microarray analysis can detect a large number of viruses simultaneously. The weak point of the microarray assay is its low sensitivity and specificity [Wang et al., 2002].

It has been demonstrated that many viruses are associated with human diseases, and such human pathogenic viruses include both DNA and RNA viruses. An ideal virus screening system may be a system capable of detecting all the human pathogenic viruses simultaneously. In the present study, a real-time PCR system capable of detecting more than one hundred human viruses in a 96-well reaction plate simultaneously was established, designated as the "multivirus real-time PCR" system. In this system, two viruses are detected in one well using a duplex TaqMan real-time reverse transcriptase (RT)-PCR system; since more than 82 different duplex real-time PCRs are performed in a 96-well plate except wells for standard curve and

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: Ministry of Health, Labor and Welfare (Health and Labor Sciences Research Grants; partial support); Grant sponsor: Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant sponsor: Japan Health Sciences Foundation (Research on Publicly Essential Drugs and Medical Devices).

*Correspondence to: Harutaka Katano, Department of Pathology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku, Tokyo 162-8640, Japan. E-mail: katano@nih.go.jp

Accepted 2 September 2010

DOI 10.1002/jmv.21962

Published online in Wiley Online Library (wileyonlinelibrary.com).

© 2010 WILEY-LISS, INC.

internal controls, theoretically 163 human viruses can be detected in a 96-well plate simultaneously. Using this system, the distribution and quantification of viruses were investigated in organ specimens from autopsies of 20 acquired immunodeficiency syndrome (AIDS) patients. In addition, clinical samples from patients with uncertain diagnoses were examined to identify the causes of infection.

MATERIALS AND METHODS

Probe–Primer Sets

A total of 163 human viruses were selected as targets (Table I). The choice of the viruses was based on their associations with human diseases, prevalence among humans, and possibility of the usages as vectors to human cells. Probe–primer sets for each virus were designed using Primer Express 2.0 (Applied Biosystems, Foster City, CA) (Supplementary Table I). Probe–primer sets published elsewhere were employed for some of the viruses. Probes and primers were synthesized by Sigma Genosys (Sigma–Aldrich, St. Louis, MO). Probes were labeled with 6-carboxyfluorescein (FAM)—6-carboxytetramethylrhodamine (TAMRA) or hexacholoro-6-carboxyfluorescein (HEX)—non-fluorescent Black Hole Quencher (BHQ)-1. Each probe–primer set was confirmed to react with at least 10 copies of a positive control plasmid containing each virus fragment, using conventional TaqMan real-time PCR (Applied Biosystems).

Establishment of Multivirus Real-Time PCR

A duplex TaqMan real-time RT-PCR system was designed to detect many viruses in a 96-well plate. Design of this system, designated as the "multivirus real-time PCR," is shown in Figure 1A. Quantitect Multiplex Probe RT-PCR kit (Qiagen, Hilden, Germany), MicroAmp Optical 96-Well Reaction Plates (Applied Biosystems), and MicroAmp Optical Adhesive Film (Applied Biosystems) were used as 2× master mix, 96-well plates, and adhesive film, respectively. Each well contains two probe–primer sets with 6-FAM- and HEX-labeled probes, allowing two viruses were to be detected in each well, and the 163 viruses listed in Table I to be detected in a 96-well plate simultaneously. A standard curve was established for nine wells of each plate (A1–A9), which contained FAM- or HEX-labeled probes and primers for green fluorescent protein and glutathione *S*-transferase genes with control plasmids at 10^1 to 10^7 copies. Thus, an approximate copy number of each virus could be calculated based on the standard curve. To use the system routinely, 2× probe–primer mix was stored in a 96-well plate at −20°C. For detection of viruses, DNA and RNA samples (50 ng per well) were added to 2× master mix with (for RNA) and without (for DNA) RT. When sufficient amounts of DNA or RNA were not obtained from clinical samples, <50 ng of DNA or RNA per well were applied in this system. Ten microliters of 2× probe–primer mix and 10 µl of 2× master mix with sample DNA (36 wells) or RNA (60 wells) were then

TABLE I. List of Target Viruses

DNA virus
Polyomavirus: JC virus, BK virus, Simian virus 40
Papillomavirus: Human papillomavirus 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73
Parvovirus: Adeno-associated virus 1, 2, 3, 5; Parvovirus B19; human bocavirus; adenovirus A, B, C, D, E, F
Herpes virus: Human herpesvirus 1–8, B virus
Poxvirus: Variola virus, Monkey pox virus, Molluscum contagiosum virus
Anellovirus: Torque teno virus
Hepadnavirus: Hepatitis B virus
Other: Mimivirus
RNA virus
Filovirus: Ebola virus, Marburg virus
Bunyavirus: Crimean–Congo hemorrhagic fever virus, hemorrhagic fever with renal syndrome virus (Hantaan, Dovrava, Puumala, and Seoul), Rift valley fever virus, Sin Nombre virus
Arenavirus: Lassa virus, Junin, Guanarito, Machupo, Sabia
Togavirus: Equine encephalitis virus (Venezuelan, Eastern, and Western), Sindbis virus, Mayaro virus, Getah virus, Chikungunya virus, Rubella virus
Enterovirus: Enterovirus 68, 71; Poliovirus 1,2,3; Coxsackievirus A2, A3, A4, A5, A6, A8, A9, A10, A16, A21, A24, B1, B2, B3, B4, B5, B6; Echovirus 5, 6, 7, 9, 11, 13, 14, 16, 17, 18, 25, 30; Parechovirus 1, 3; Rhinovirus A, B; rotavirus; reovirus 1–4; Melaka virus; Colorado tick borne fever virus
Flavivirus: Dengue virus 1, 2; Japanese encephalitis virus; Murray Valley encephalitis virus; St. Louis encephalitis virus; West Nile virus; Tick-borne encephalitis virus; Yellow fever virus
Orthomyxovirus: Influenza virus A, B, C; H5N1
Paramyxovirus: Parainfluenza virus 1–3; Hendra virus; Mumps virus; Measles virus; Sendai virus; RS virus A, B; metapneumovirus; Nipah virus
Rabdovirus: Rabies virus; Lyssavirus 5, 6; Chandipura virus; Duvenhage virus
Coronavirus: Coronavirus OC43, 229E, NL63, SARS virus
Calicivirus: Sapovirus, Norwalk-like virus 1, 2
Hepatitis virus: Hepatitis A virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, GB virus
Retrovirus: human immunodeficiency virus 1; human T cell leukemia virus 1, 2; human endogenous retrovirus K, H, W
Other: Astrovirus, Borna disease virus

Biosystems) or an Mx3005P (Stratagene, La Jolla, CA). The RT-PCR conditions were 50°C for 30 min and 95°C for 15 min, followed by 40 cycles of 94°C for 15 sec, and 60°C for 1 min. Quantitative results of viruses were obtained by generating standard curves for two plasmids in the A1–A9 wells. Real-time PCR using a condition for RT-PCR (50°C for 30 min and 95°C for 15 min, followed by 40 cycles of 94°C for 15 sec and 60°C for 1 min) had similar sensitivity to real-time PCR using usual DNA conditions (95°C for 5 min, followed by 40 cycles of 94°C for 15 sec and 60°C for 1 min) in the detection for some DNA viruses (Supplementary Fig. 1A). In addition, the duplex real-time PCR using Quantitect Multiplex Probe RT-PCR kit (Qiagen) had similar sensitivity to single real-time PCR procedures using Quantitect Probe RT-PCR kit (Qiagen) in several probe–primer sets (Supplementary Fig. 1B).

Gene Expression Image

A gene expression image was produced with TreeView and Cluster software by Michael Eisen, University of California at Berkeley (http://rana.lbl.gov/EisenSoftware.htm) [Eisen et al., 1998].

Determination of the Positivity and Copy Numbers of Viruses

The positivity and virus titer of all positive samples were confirmed with individual standard real-time (RT-) PCR systems using the same probe–primer sets. Virus DNA copy numbers per cell were calculated by dividing virus DNA copy numbers by half of beta-actin copy numbers, since each cell has two copies of DNA in two alleles [Asahi-Ozaki et al., 2006].

Patients and Samples

The study protocol was approved by the Institutional Review Board, National Institute of Infectious Diseases, Japan (Approval No. 156). Tissues were taken at autopsy from various organs of 20 patients with AIDS. All tissues were frozen immediately, and stored at −80°C. The clinical information of the patients is summarized in Table II. A total of 19 patients were male. The mean age of the patients was 41.8 years (range: 19–67 years), and the mean of CD4 counts was 17 cells/μl (range: 0–241). Risk factors for HIV infection in the patients were men who had sex with men (10), heterosexuality (5), and hemophilia (5). At least seven patients had lymphoma, and two had Kaposi's sarcoma. No patients received highly active anti-retroviral therapy (HAART). In addition, 40 clinical samples from patients with uncertain diagnoses were investigated (Table III). These clinical samples were sent to our department for virus diagnosis. Informed consents were obtained by the clinical doctors. Positive control DNA or RNA samples extracted from virus-infected cells or tissues were kindly provided by many researchers in National Institute of Infectious Diseases (Supplementary Table II).

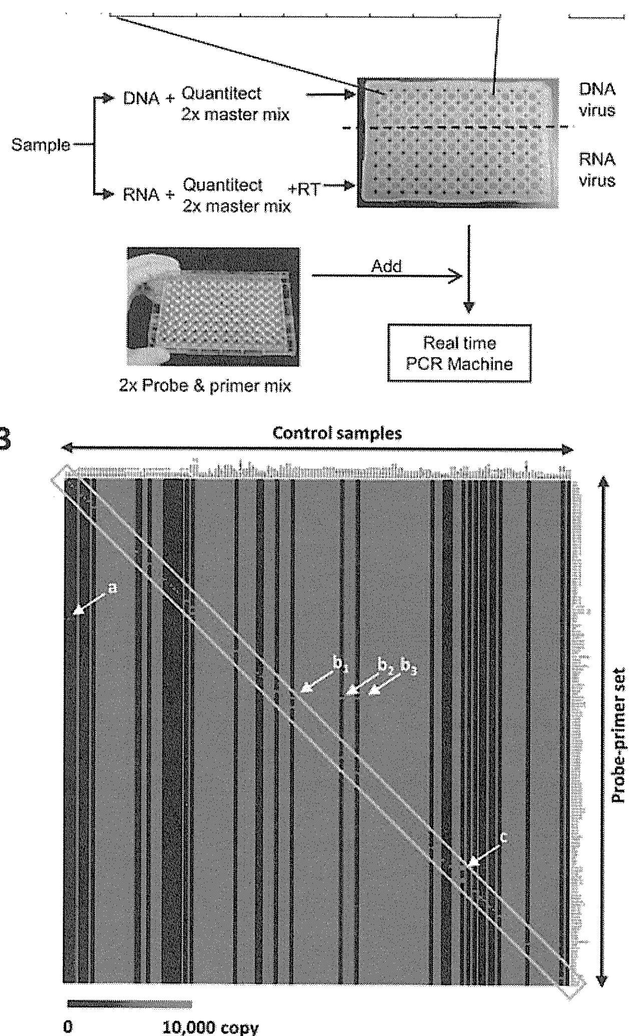


Fig. 1. Establishment and validation of multivirus real-time PCR. **A**: Procedure of the multivirus real-time PCR system. DNA sample was mixed with Quantitect 2× master mix, and RNA sample was mixed with Quantitect 2× master mix and reverse transcriptase (RT) mix. These mixtures were poured into each well in a 96-well plate at 10 μl per well. Ten microliters of 2× probe and primer mix were then added to each well in a premixed 96-well plate. Finally, the virus genes were amplified and detected in a real-time PCR machine for 2 hr. **B**: Validation of the multivirus real-time PCR. A gene expression image by TreeView software based on the results of the multivirus real-time PCR for control samples is shown. A horizontal line shows each probe–primer set and a vertical line is one sample of positive control. Gray vertical lines indicate no sample. A scale bar indicates copy number of color. A green box indicates specific reactions of target positive controls in specific probe–primer sets. Arrows of (a–c) also show specific signals. The arrow (a) shows positive signal for TTV in a brain sample with both JCV and TTV infection. The arrows (b1–3) show that a probe–primer set for pan-enterovirus reacted with poliovirus (b1), Coxsackievirus B3 (b2), and Echovirus 6 (b3) positive samples. The arrow (c) shows that a probe–primer set for influenza virus A reacted with H5N1 influenza virus. Details of positive controls were listed in Supplementary Table II.

Age	Sex	Risk factor	Complications	CD4[a]	Detected viruses by multivirus real-time PCR
49	M	MSM	PCP, aspergillus	NT	TTV, HBV, HERV-H
37	M	MSM	CMV, toxoplasma, PCP	NT	HSV-1, CMV, TTV, HIV-1, HERV-H
29	M	Drug	PCP	1	JCV, Adv-B, HSV-1, EBV, CMV, HHV-6, HHV-7, TTV, HBV, HIV-1, HERV-H
37	M	Blood product	MAC, CMV	1	EBV, CMV, HHV-6, TTV, HIV-1, HERV-H
43	M	Heterosexual	ML, CMV, cryptococcus	5	B19, Adv-A, EBV, CMV, HHV-6, TTV, HIV-1, HERV-H
54	M	MSM	CMV	NT	EBV, CMV, HHV-6, TTV, HBV, HIV-1, HERV-H
33	M	MSM	CMV	4	B19, CMV, HHV-6, TTV, HIV-1, HERV-H
47	M	MSM	HIV-encephalitis, KS, ML	1	BKV, CMV, HHV-6, TTV, HIV-1, HCV
35	M	Blood product	CMV	1	B19, CMV, TTV, HIV-1, HERV-H
27	F	Heterosexual	ML, MAC, CMV	3	BKV, EBV, CMV, TTV, HIV-1, HERV-H
50	M	MSM	HIV-encephalitis, ML, cryptococcus, CMV	0	B19, CMV, HHV-6, TTV, HIV-1, HERV-H
19	M	Blood product	HIV-encephalitis	2	BKV, TTV, HIV-1, HERV-H
26	M	Blood product	PML	3	JCV, BKV, B19, Adv-B, EBV, HHV-6, HHV-7, TTV, Echo6, HCV, HERV-H
67	M	MSM	ML	241	JCV, CMV, HHV-6, TTV, HIV-1, HERV-H
62	M	MSM	CMV, PCP	4	AAV-2, B19, EBV, CMV, HHV-6, TTV, HIV-1, HERV-H
28	M	Blood product	CMV, MAC	3	JCV, BKV, B19, CMV, HHV-7, TTV, HBV, HIV-1, HERV-H
46	M	Heterosexual	ML, aspergillus, CMV	5	BKV, AAV-2, B19, Adv-D, EBV, CMV, HHV-6, TTV, RSV-B, HIV-1
47	M	MSM	PEL, CMV	7	JCV, B19, EBV, CMV, HHV-6, HHV-8, TTV, HIV-1, HERV-K, HERV-H
60	M	MSM	ML, CMV, KS	1	B19, CMV, TTV, HIV-1, HERV-H
40	M	Heterosexual	CMV	NT	BKV, AAV-2, CMV, HHV-6, HHV-7, TTV, HBV, HERV-H

:no-associated virus; Adv, adenovirus; B19, parvovirus B19; BKV, BK virus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HBV, B virus; HCV, hepatitis C virus; HERV, human endogenous retrovirus; HHV, human herpesvirus; HIV-1, human immunodeficiency ISV, herpes simplex virus; JCV, JC virus; KS, Kaposi's sarcoma; MAC, mycobacterium avium-intracellulare complex; ML, malignant a; MSM, Men who have sex with men; NT, not tested; PCP, Pneumocystis pneumonia; PML, progressive multifocal leukoencephal- :SV, respiratory syncytial virus; TTV, Torque teno virus.
nts per μl.

DNA and RNA Extraction

ɔic acid extraction methods differed according ;ype of samples. Each frozen tissue sample was in two, one part for DNA extraction and another A extraction. For DNA extraction, the samples omogenized with Multi-Beads Shocker (Yasui Tokyo, Japan) in TEN buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0, and 100 mM NaCl) with ml proteinase K and 0.1% sodium dodecyl DNA was extracted from the homogenized using the phenol–chloroform method. Total 'as extracted from frozen tissues using Isogen ı Gene, Tokyo, Japan). The samples were homogenized in the Isogen with Multi-Beads Shocker, and the extraction was performed according to the manufacturer's instructions. For small samples including tissue biopsy, blood, serum and cerebral fluid, both DNA and RNA were extracted simultaneously with All Prep Kit (Qiagen). All RNA samples were treated with DNase (Turbo DNA-Free, Ambion, Austin, TX) for 20 min according to the manufacturer's instructions.

RESULTS

Validation of Multivirus Real-Time PCR

To validate the sensitivity and specificity of each probe and primer set used in the system, DNA or RNA samples

TABLE III. Identification of Pathogenic Virus in Clinical Samples From Patients With Uncertain Diagnoses

;	n	Samples	Identified pathogens (cases)
;s	9	Liver biopsy	Parvovirus B19 (2), HHV-6 (3), TTV (2)
ılitis	11	Brain biopsy, serum, cerebral fluids	HSV-1 (2), HHV-6 (1), parechovirus 3 (1)
litis	6	Heart autopsy	Parvovirus B19 (1), TTV (2)
death	4	Blood, serum	TTV (1)
	10	Tissue, blood, serum	Parvovirus B19 (1), EBV (1), CMV (1), HHV-7 (1), TTV (2)
	40	—	—

ıl viruses in the cases are underlined.

extracted from virus-infected cells, supernatants, body fluids, or tissues were examined in this multivirus real-time PCR system (Supplementary Table II). Each probe and primer set amplified a gene fragment of target virus specifically (Fig. 1B and Supplementary Table II). When using supernatants of virus-infected cells, the reactions were specific. Some samples of supernatants were positive for two or three viruses, because the target virus belonged to several categories. For example, the RNA sample extracted from supernatants of H5N1 influenza virus-infected cells was positive in the wells with probe and primer sets for both H5N1 influenza virus and influenza-A virus. Some clinical samples, such as pathological samples and body fluids, were positive for other viruses as well as target viruses because of the presence of such viruses in the samples (Supplementary Table II and Fig. 1B, arrow (a)). Although not all positive control samples could be collected, the results confirmed the adequate specificity of each probe–primer set for its target virus for virus screening. The multivirus real-time PCR system also detects human internal control genes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH, DNA, and mRNA), beta-actin (DNA), and beta-2-microglobulin (mRNA) (Supplementary Tables I and II). It is known that certain specimen such as serum may have inhibitory effects on PCR [Vandenvelde et al., 1993; Willems et al., 1993]. Copy numbers of internal controls would be informative to know cell numbers and inhibitory effect by the sample.

Detection of Viruses in AIDS Autopsies

Using the multivirus real-time PCR, the presence of viruses was investigated in 20 AIDS autopsies. The multivirus real-time PCR detected 15 DNA viruses: JC virus (JCV), BK virus (BKV), adeno-associated virus (AAV)-2, parvovirus B19, three subgroups of adenovirus (A, B, and D), herpes simplex virus 1 (HSV-1), Epstein–Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus (HHV)-6, -7, -8, TT virus (TTV), and

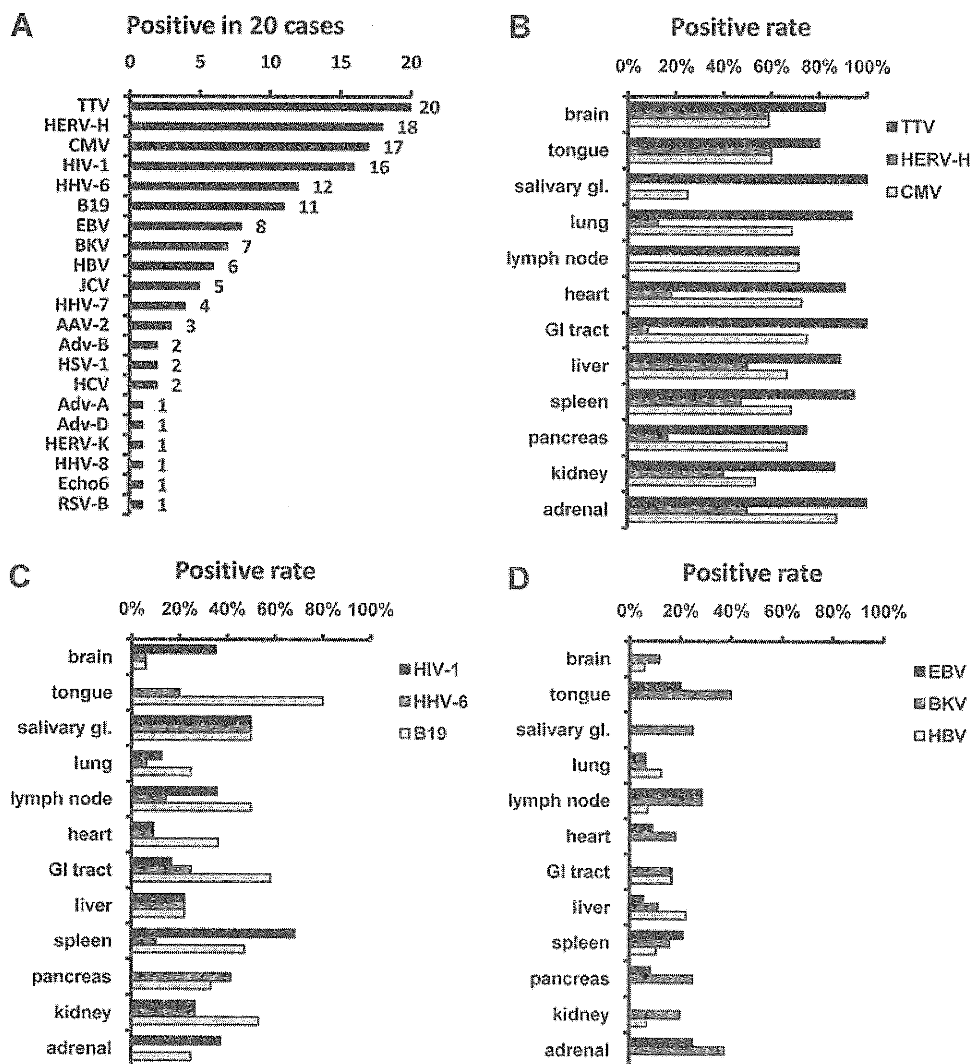


Fig. 2. Viruses detected in autopsied organs of AIDS patients. **A**: Positive number of each virus in the 20 cases of AIDS autopsy. **B–D**: Positive rates of CMV, HIV-1, and HHV-6 in organs. GI, gastrointestinal.

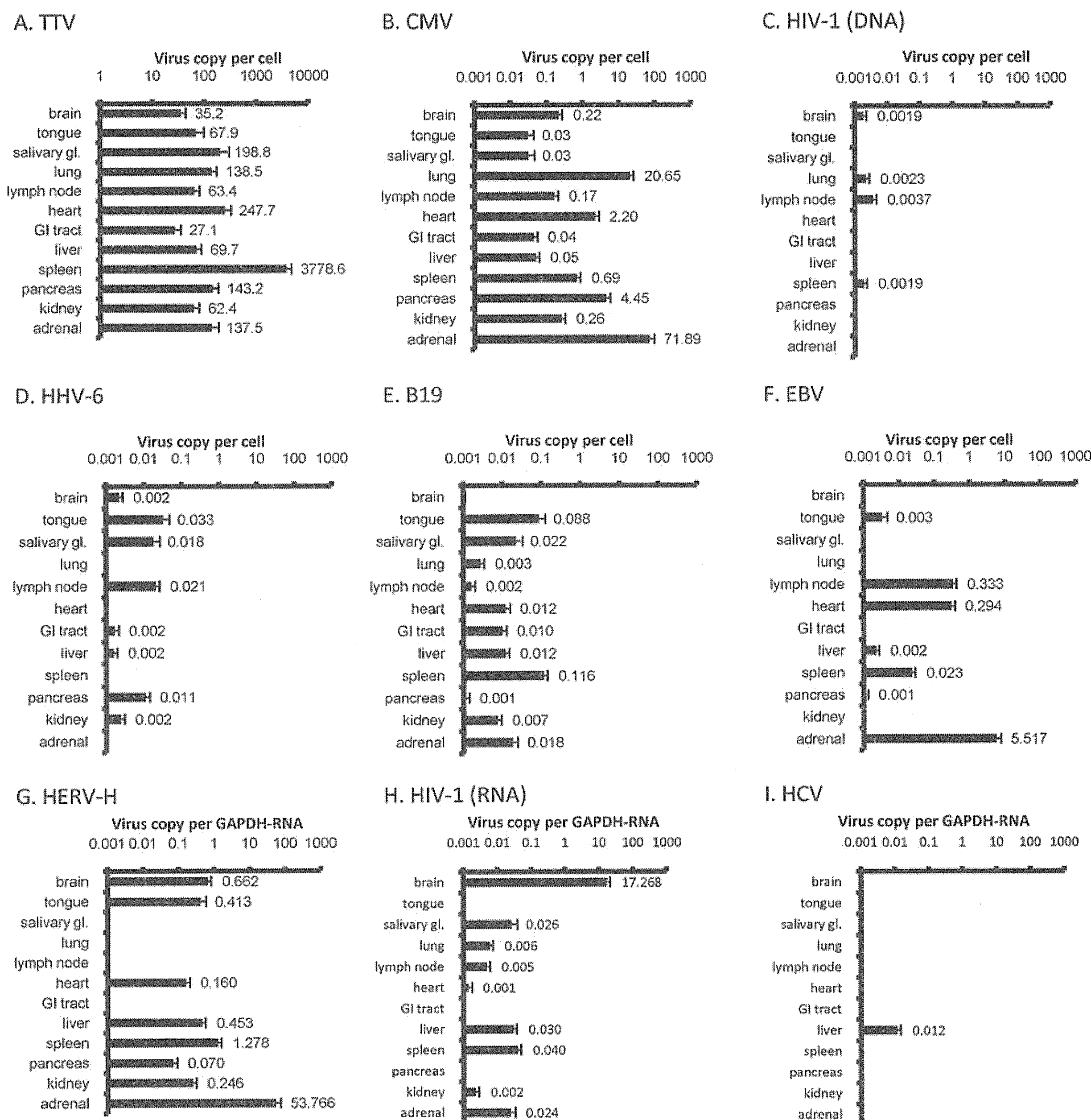


Fig. 3. Mean values of virus copy numbers in organs. Mean of copy numbers per cells are shown in DNA samples (**A–F**). Mean ratios of virus copy number per hGAPDH-RNA copy number are shown in RNA samples (**G–I**). Error bars show standard errors. One brain sample contained HIV-associated encephalopathy (C,H), and one of the heart and adrenal sample contained EBV-associated lymphoma (F).

hepatitis B virus (HBV). It also detected six RNA viruses: echovirus 6, respiratory syncytial (RS) virus type B, hepatitis C virus (HCV), HIV-1, and human endogenous retrovirus (HERV)-H and -K, in 20 cases of AIDS autopsies (Fig. 2). A few other viruses were detected at low copies in some samples, but additional individual standard real-time (RT-) PCR systems using the same probe–primer sets showed negative results, indicating that they were false positive. Although HIV-1 infections were confirmed clinically in all the patients, HIV-1 was not detected in four of the autopsy cases, even using both DNA and RNA samples. TTV, HERV-H, CMV, HIV, HHV-6, parvovirus B19, EBV, BKV, and HBV were detected in many organs, suggesting broad distribution (Fig. 2B–D). On the other hand, the positive rate of each virus differed among organs. CMV was detected most frequently in the adrenal gland, but HIV-1 was most common in the spleen, HHV-6 in the salivary gland, and HBV in the liver.

The multivirus real-time PCR also revealed copy numbers of each virus in AIDS autopsy (Fig. 3). High numbers of TTV copies were detected frequently in various organs without any symptoms, suggesting a ubiquitous distribution in the samples and no associa-

tion with any specific diseases (Fig. 3A). High numbers of CMV copies were detected in adrenal gland, lung, and pancreas (Fig. 3B). To confirm the results of the real-time PCR, CMV positivity was investigated using inclusion bodies in the pathological samples. Inclusion bodies of CMV were detected frequently in the adrenal gland, pancreas, and lung of AIDS autopsy cases (Supplementary Fig. 2). These results correlated with those of the real-time PCR. HHV-6 and parvovirus B19 showed low copy numbers in all the organs tested (Fig. 3D,E). High numbers of EBV copies were detected in the heart and adrenal gland, as well as lymph node and spleen (Fig. 3F). However, the heart and adrenal samples included lesions of EBV-associated lymphomas. Thus, EBV was detected in the lymphomas in those samples. The lymphomas in adrenal glands also included high numbers of HERV-H copies, affecting the results of HERV-H copy numbers in adrenal glands (Fig. 3G). High numbers of HIV-1-RNA copies, but not HIV-1-DNA, were also found in the brain of one case with HIV-1 encephalopathy (Fig. 3C,H). HCV was detected only in the liver of two patients (Fig. 3I).

Identification of Virus in Clinical Samples From Patients With Uncertain Diagnoses

Using the multivirus real-time PCR, clinical samples from 40 patients with uncertain diagnoses were examined to identify their causes of infection (Table III). The multivirus real-time PCR system identified HSV-1, HHV-6, or parechovirus 3 as a possible cause of infection in 4 out of 11 patients with encephalitis. HSV-1 was identified in brain biopsy tissues from two patients with encephalitis. A high copy number of HHV-6 was detected in the serum of a patient (1.5×10^7 copies/ml in the serum). In another patient, parechovirus 3 were detected in cerebral fluids. The presence of these viruses in the samples was confirmed by individual real-time PCR specific for each virus, and conventional (RT-) PCR. Clinical manifestations of these four patients were compatible with the virus infections. In addition, 29 samples from patients with other diseases such as myocarditis, hepatitis, and sudden death were examined. Parvovirus B19, EBV, CMV, HHV-6, HHV-7, and TTV were detected in the samples; however, the titers of these viruses were low. In addition, immunohistochemistry and in situ hybridization could not detect the viruses in the samples. It was therefore concluded that these viruses were not the causes of diseases in the cases.

DISCUSSION

In the present study, a new real-time PCR system was developed, designated as the "multivirus real-time PCR," that had the potential to detect 163 viruses simultaneously. This multivirus real-time PCR can detect 47 DNA viruses and 116 RNA viruses on a 96-well plate theoretically. This system revealed the anatomical distributions of human pathogenic viruses in AIDS autopsy cases. In addition, viruses were identified in four cases of encephalitis as the cause of infection. This multivirus real-time PCR system could be a useful technique for detection of virus in specimens from patients with uncertain diagnoses.

Real-time PCR is a sensitive detection system for the diagnosis of virus infection. TaqMan PCR has a high specificity compared with other systems because of its specific fluorescence probes. In addition, recent multiplex fluorescence technology is able to detect several genes in each tube without non-specific cross-reactions. Since one-step real-time RT-PCR employs specific primers as RT primers, with targets shorter than 100 bp, this system can detect short fragments of RNA viruses specifically with high sensitivity. The sensitivity and specificity of this system are equivalent to those of standard real-time PCR systems (Supplementary Fig. 1), and its sensitivity would be much higher than in microarray systems. In addition, the multivirus real-time PCR system requires only 5 μg each of DNA and RNA for detecting 163 viruses.

One disadvantage of this system is cost. To establish this system, 176 probe–primer sets should be prepared. Moreover, about 1 ml of Quantitect 2× master mix was used in single reaction of 96-well plate, which costs about 25,000 yen (approximately 263 U.S. dollars; containing probe–primer sets: ¥36 × 176 sets = ¥6,336, Quantitect 2× master mix: ¥16,000, filtered tips, 96-well reaction plate and seal: ¥2,664) per sample in a 96-well plate reaction to test. However, once the system is established, the procedure is very easy and takes 3 hr to obtain the results. Thus, the newly developed multivirus real-time PCR could be a useful tool for detecting pathogens in specimens from patients with uncertain diagnoses.

There is little current information about quantification of pathogenic viruses in immunocompromised hosts. Chen and Hudnall [2006] described anatomical mapping of herpes viruses in eight autopsy cases, including two AIDS cases. The multivirus real-time PCR showed that 21 of the 163 probe–primer sets for virus produced positive reactions in AIDS autopsy samples. Many RNA viruses were negative in all cases. Although the low detection rate of RNA viruses might be associated with the quality of extracted RNA, these results suggest that the AIDS patients in the present study were infected with limited types of viruses. TTV and HHV-6 were detected frequently in AIDS autopsy samples and some clinical samples. TTV was identified from a hepatitis patient as a hepatitis-associated virus [Nishizawa et al., 1997]. However, TTV, a ubiquitous virus, was shown to be present in various tissues [Okamoto, 2009]. Although TTV titers were relatively high compared with those of other viruses, broad TTV distribution suggests that it is not associated with specific diseases in immunocompromised hosts. HHV-6 is another ubiquitous virus with which almost 100% of adults are infected. Primary infection of HHV-6 causes exanthema subitum in children [Yamanishi et al., 1988]. Reactivation of HHV-6 may cause hepatitis, pneumonia, and encephalitis in immunocompromised hosts, such as

transplant patients [Ljungman, 2002]. The average number of HHV-6 copies in the HHV-6-positive samples in AIDS autopsy was 0.008 copies per cell, suggesting low numbers of HHV-6 copies in the organs. On the other hand, HHV-6 was identified as a possible cause of infection in a clinical case of encephalitis because of extremely high numbers of copies in the serum and clinical manifestations [Ogata et al., 2010]. Thus, a virus's copy number is important information to estimate its etiology. CMV was frequently detected in AIDS autopsy samples by the multivirus real-time PCR system. High CMV copy numbers in the adrenal gland, pancreas and lung were associated with the occurrence of CMV-associated adrenitis, pancreatitis, and pneumonia. CMV was detected frequently in the adrenal gland [Pulakhandam and Dincsoy, 1990]. The results of CMV detection in multivirus real-time PCR were correlated highly with the frequency of CMV inclusion bodies on the slides, suggesting that the occurrence of CMV-associated diseases is associated with virus titers of CMV in organs.

The multivirus real-time PCR failed to detect HIV-1 DNA or RNA in 4 cases out of 20 AIDS autopsies. There are several possible explanations for these results. Although none of the patients received HAART, HIV-1 titers always change in AIDS patients [Ho et al., 1989]; the autopsy samples might have had insufficient HIV-1 titers for detection by real-time PCR. Also, the probe and primers used in this system might not detect the HIV-1 because of mutations in the target regions. Mutations in HIV-1 occur so frequently that it is difficult to detect HIV-1 using one or two probe–primer sets [Desire et al., 2001; Yun et al., 2002].

Consequently, a multivirus real-time PCR system with the potential to detect 163 viruses simultaneously has been established in the present study. Although the system has some disadvantages with regard to cost and procedure, it will be a powerful tool for virus screening of clinical samples in laboratories. Since it is relatively easy to change probe–primer sets in the 96-well plate, it is possible for this system to change detectable viruses, implying that a new system detecting new viruses can be established quickly. Future refinement of its operation, such as higher throughput and microfluid techniques, may resolve the disadvantages of this system.

ACKNOWLEDGMENTS

We thank Mr. Kouta Sakamoto and Ms. Yuko Sato for technical assistant and Dr. Tadahito Kanda, Dr. Seiichiro Mori, Dr. Takato Odagiri, Dr. Atsushi Kato, Dr. Katsuhiro Komase, Dr. Fumihiro Taguchi, Dr. Kei Numazaki, Dr. Shigeru Morikawa, Dr. Tomohiko Takasaki, Dr. Naoki Inoue, Dr. Tetsuro Suzuki, Dr. Naokazu Takeda, Dr. Tetsuo Yoneyama, Dr. Hiroyuki Shimizu, Dr. Haruko Shirato, Dr. Toshihiko Matsukura, Dr. Asato Kojima, Dr. Hidehiro Takahashi, Dr. Kenzo Tokunaga, Dr. Michiko Tanaka, Dr. Kiyoko Ogawa-Goto, and Dr. Yasuko Asahi-Ozaki of the National Institute of Infectious Diseases for providing positive controls for RNA or DNA extracted from virus-infected cells.

REFERENCES

Asahi-Ozaki Y, Sato Y, Kanno T, Sata T, Katano H. 2006. Quantitative analysis of Kaposi sarcoma-associated herpesvirus (KSHV) in KSHV-associated diseases. J Infect Dis 193:773–782.

Bellau-Pujol S, Vabret A, Legrand L, Dina J, Gouarin S, Petitjean-Lecherbonnier J, Pozzetto B, Ginevra C, Freymuth F. 2005. Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses. J Virol Methods 126:53–63.

Chen T, Hudnall SD. 2006. Anatomical mapping of human herpesvirus reservoirs of infection. Mod Pathol 19:726–737.

Desire N, Dehee A, Schneider V, Jacomet C, Goujon C, Girard PM, Rozenbaum W, Nicolas JC. 2001. Quantification of human immunodeficiency virus type 1 proviral load by a TaqMan real-time PCR assay. J Clin Microbiol 39:1303–1310.

Eisen MB, Spellman PT, Brown PO, Botstein D. 1998. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 95:14863–14868.

Ho DD, Moudgil T, Alam M. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med 321:1621–1625.

Li H, McCormac MA, Estes RW, Sefers SE, Dare RK, Chappell JD, Erdman DD, Wright PF, Tang YW. 2007. Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections. J Clin Microbiol 45:2105–2109.

Ljungman P. 2002. Beta-herpesvirus challenges in the transplant recipient. J Infect Dis 186:S99–S109.

Mahony J, Chong S, Merante F, Yaghoubian S, Sinha T, Lisle C, Janeczko R. 2007. Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of multiplex PCR and a fluid microbead-based assay. J Clin Microbiol 45:2965–2970.

Molenkamp R, van der Ham A, Schinkel J, Beld M. 2007. Simultaneous detection of five different DNA targets by real-time Taqman PCR using the Roche LightCycler480: Application in viral molecular diagnostics. J Virol Methods 141:205–211.

Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. 1997. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun 241:92–97.

Nolte FS, Marshall DJ, Rasberry C, Schievelbein S, Banks GG, Storch GA, Arens MQ, Buller RS, Prudent JR. 2007. MultiCode-PLx system for multiplexed detection of seventeen respiratory viruses. J Clin Microbiol 45:2779–2786.

Ogata M, Satou T, Kawano R, Takakura S, Goto K, Ikewaki J, Kohno K, Ikebe T, Ando T, Miyazaki Y, Ohtsuka E, Saburi Y, Saikawa T, Kadota J. 2010. Correlations of HHV-6 viral load and plasma IL-6 concentration with HHV-6 encephalitis in allogeneic stem cell transplant recipients. Bone Marrow Transplant 45:129–136.

Okamoto H. 2009. History of discoveries and pathogenicity of TT viruses. Curr Top Microbiol Immunol 331:1–20.

Pulakhandam U, Dincsoy HP. 1990. Cytomegaloviral adrenalitis and adrenal insufficiency in AIDS. Am J Clin Pathol 93:651–656.

Storch GA. 2000. Diagnostic virology. Clin Infect Dis 31:739–751.

van de Pol AC, van Loon AM, Wolfs TF, Jansen NJ, Nijhuis M, Breteler EK, Schuurman R, Rossen JW. 2007. Increased detection of respiratory syncytial virus, influenza viruses, parainfluenza viruses, and adenoviruses with real-time PCR in samples from patients with respiratory symptoms. J Clin Microbiol 45:2260–2262.

Vandenvelde C, Scheen R, Defoor M, Duys M, Dumon J, Van Beers D. 1993. Suppression of the inhibitory effect of denatured albumin on the polymerase chain reaction by sodium octanoate: Application to routine clinical detection of hepatitis B virus at its infectivity threshold in serum. J Virol Methods 42:251–263.

Vet JA, Majithia AR, Marras SA, Tyagi S, Dube S, Poiesz BJ, Kramer FR. 1999. Multiplex detection of four pathogenic retroviruses using molecular beacons. Proc Natl Acad Sci USA 96:6394–6399.

Wada K, Mizoguchi S, Ito Y, Kawada J, Yamauchi Y, Morishima T, Nishiyama Y, Kimura H. 2009. Multiplex real-time PCR for the simultaneous detection of herpes simplex virus, human herpesvirus 6, and human herpesvirus 7. Microbiol Immunol 53:22–29.

Wang D, Coscoy L, Zylberberg M, Avila PC, Boushey HA, Ganem D, DeRisi JL. 2002. Microarray-based detection and genotyping of viral pathogens. Proc Natl Acad Sci USA 99:15687–15692.

Willems M, Moshage H, Nevens F, Fevery J, Yap SH. 1993. Plasma collected from heparinized blood is not suitable for HCV-RNA detection by conventional RT-PCR assay. J Virol Methods 42: 127–130.

Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, Kurata T. 1988. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet 1:1065–1067.

Yun Z, Fredriksson E, Sonnerborg A. 2002. Quantification of human immunodeficiency virus type 1 proviral DNA by the TaqMan real-time PCR assay. J Clin Microbiol 40:3883–3884.

Supplementary Figure 1

A

GAPDH-DNA

Rt
non-RT

Ct

40
35
30
25
20

10 100 1000 10000 100000
Copies

B

GAPDH-mRNA

single
multiplex

Ct

38
33
28
23
18

10 100 1000 10000 100000
Copies

JCV

40
35
30
25
20

10 100 1000 10000 100000
Copies

Poliovirus

40
35
30
25
20

10 100 1000 10000 100000
Copies

HSV-1

40
35
30
25
20

10 100 1000 10000 100000
Copies

Entrovirus 71

40
35
30
25
20

10 100 1000 10000 100000
Copies

HHV-8

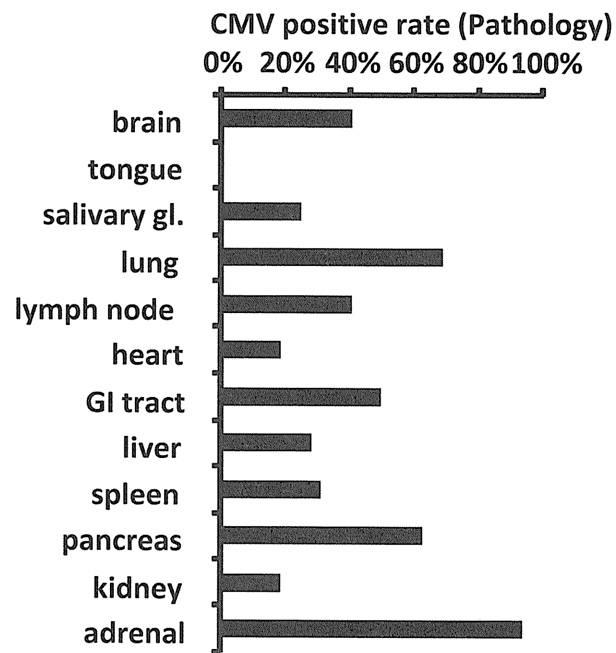
40
35
30
25
20

10 100 1000 10000 100000
Copies

EEEV

40
35
30
25
20

10 100 1000 10000 100000
Copies


CMV positive rate (Pathology)
0%
20%
40%
60%
80%
100%
brain
tongue
salivary gl.
lung
lymph node
heart
GI tract
liver
spleen
pancreas
kidney
adrenal

Journal of Medical Virology 82:400–406 (2010)

Genotypic and Clinicopathological Characterization of Kaposi's Sarcoma-Associated Herpesvirus Infection in Japan

Takayuki Kanno, Yuko Sato, Tomoyuki Nakamura, Kouta Sakamoto, Tetsutaro Sata, and Harutaka Katano*

Department of Pathology, National Institute of Infectious Diseases, Shinjuku, Tokyo, Japan

Kaposi's sarcoma-associated herpesvirus (KSHV) is related causally to Kaposi's sarcoma, primary effusion lymphoma, and a subset of cases of multicentric Castleman's disease. As the numbers of acquired immunodeficiency syndrome (AIDS) patients have increased, KSHV-associated diseases have also increased in Japan. Sporadic cases of classic Kaposi's sarcoma have also been reported in Japan. In the present study, the clinicopathological characteristics of 75 samples, comprising 68 cases of Kaposi's sarcoma, 5 cases of primary effusion lymphoma, and 5 cases of multicentric Castleman's disease were investigated. All of these cases were positive for KSHV by immunohistochemistry or PCR analysis. All fifty-two of the AIDS-associated Kaposi's sarcoma cases were males, whereas 7 of the 13 non-AIDS-associated Kaposi's sarcoma cases were females. The mean age of patients with AIDS-associated Kaposi's sarcoma or primary effusion lymphoma was 46 years, whereas the mean age of patients with non-AIDS-associated Kaposi's sarcoma or primary effusion lymphoma was 71.8 and 97.5, respectively. KSHV genotypes were determined based on the sequence of variable region 1 in the *K1* gene. Genotypes A and C of KSHV were detected in both AIDS- and non-AIDS-associated Kaposi's sarcoma. Genotype A was detected more frequently in AIDS-associated cases than non-AIDS-associated cases, suggesting that genotype C is broadly distributed in Japan, and genotype A spreads among AIDS patients. Genotype D was detected only in non-AIDS-associated Kaposi's sarcoma. These data confirmed the difference between AIDS- and non-AIDS-associated KSHV diseases with regard to age of onset, gender, and genotypes in Japan. ***J. Med. Virol. 82:400–406, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: Kaposi's sarcoma; Kaposi's sarcoma-associated herpesvirus; AIDS; genotype

© 2010 WILEY-LISS, INC.

INTRODUCTION

Kaposi's sarcoma-associated herpesvirus (KSHV, also known as human herpesvirus-8, HHV-8) was identified from a Kaposi's sarcoma (KS) specimen by representational difference analysis in 1994 [Chang et al., 1994]. KSHV has been detected in KS, primary effusion lymphoma (PEL) and a subset of multicentric Castleman's disease (MCD) cases [Moore and Chang, 2001]. KS was first described in 1872 by Moriz Kaposi, a Hungarian dermatologist, as an idiopathic, multipigmented sarcoma of the skin [Kaposi, 1872]. KS is classified into four types: classic, endemic, iatrogenic, and acquired immunodeficiency syndrome (AIDS)-associated KS (AIDS-KS) [Antman and Chang, 2000]. Classic KS affects typically elderly men in Mediterranean littoral, endemic KS affects typically people in Africa, iatrogenic KS affects most commonly organ-transplant recipients receiving immunosuppressive therapy and AIDS-KS is mainly associated with homosexual men infected with human immunodeficiency virus (HIV). In Japan, KS was a very rare condition prior to 1980 [Fujii et al., 1986]. A few patients with adult

The authors declare no conflict of interest.

Grant sponsor: Japanese Health Sciences Foundation (Research on the Publicly Essential Drugs and Medical Devices, partial support to TK, TS, and HK); Grant number: SAA4832; Grant sponsor: Ministry of Health, Labor and Welfare (Health and Labor Sciences Research Grants on Measures for HIV/AIDS to HK); Grant number: H21-AIDS-Ippan-006; Grant sponsor: Ministry of Health, Labor and Welfare (Intractable Diseases to TS); Grant number: H20-Nanchi-Ippan-035; Grant sponsor: Ministry of Health, Labor and Welfare (Emerging and Re-emerging Infectious Diseases to TS and HK); Grant number: H20-Shinko-Ippan-006; Grant sponsor: Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Scientific Research to HK); Grant number: 21590520.

*Correspondence to: Harutaka Katano, DDS, Department of Pathology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku, Tokyo 162-8640, Japan.
E-mail: katano@nih.go.jp

Accepted 17 October 2009

DOI 10.1002/jmv.21715

Published online in Wiley InterScience (www.interscience.wiley.com)