developed at the same pace throughout the follow-up period, suggesting that the invasive amebiasis in these patients represented new infection rather than exacerbation of subclinical infection. The median anti-*E. histolytica* titer at the onset of invasive amebiasis in patients of high anti-*E. histolytica* titer group was not higher than that at first visit, whereas the titer increased at the onset compared with that at baseline in low anti-*E. histolytica* titer group. In addition, uni- and multivariate analyses identified high titer of anti-*E. histolytica* antibody at baseline as the only significant risk factor for future development of invasive amebiasis; seropositivity to other STIs was not a significant factor. These results add support to the aforementioned hypothesis regarding the difference in the pathology of invasive amebiasis between the high and low anti-*E. histolytica* groups. In this study, 15 asymptomatic but anti-*E. histolytica*-positive patients were treated with metronidazole at first visit (excluded from the follow-up analysis study), and none of them developed invasive amebiasis (median follow-up period, 11.7 months), suggesting the potential effectiveness of preemptive therapy for asymptomatic individuals with high anti-*E. histolytica* titer.

In conclusion, our results showed a relatively high prevalence of amebiasis in HIV-1-infected individuals in Japan, and that subclinical amebiasis is common among these individuals. The results emphasize the difficulty of disease control in not only individual patients with amebiasis but also in epidemiological control of this condition due to the long duration of subclinical infection of *E. histolytica*. Anti-*E. histolytica* testing for high-risk individuals could be helpful in early diagnosis of subclinical amebiasis, and early treatment of patients with such infection could prevent the development of invasive amebiasis and the transmission to others in the same community. Further studies to clarify the pathogenesis of invasive amebiasis are warranted.

Notes

Acknowledgments. We thank all clinical staff at the AIDS Clinical Center for their help in the completion of this study.

Financial support. This study was supported by a grant from the Ministry of Health, Labor, and Welfare of Japan (H25-promotion-general-014).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Walsh JA. Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality. Rev Infect Dis **1986**; 8:228–38.
2. Hung CC, Chang SY, Ji DD. *Entamoeba histolytica* infection in men who have sex with men. Lancet Infect Dis **2012**; 12:729–36.
3. James R, Barratt J, Marriott D, Harkness J, Stark D. Seroprevalence of *Entamoeba histolytica* infection among men who have sex with men in Sydney, Australia. Am J Trop Med Hyg **2010**; 83:914–6.
4. van Hal SJ, Stark DJ, Fotedar R, Marriott D, Ellis JT, Harkness JL. Amoebiasis: current status in Australia. Med J Aust **2007**; 186:412–6.
5. Chen YM, Kuo SH. HIV-1 in Taiwan. Lancet **2007**; 369:623–5.
6. Lee JH, Kim GJ, Choi BS, et al. Increasing late diagnosis in HIV infection in South Korea: 2000–2007. BMC Public Health **2010**; 10:411.
7. van Griensven F, de Lind van Wijngaarden JW. A review of the epidemiology of HIV infection and prevention responses among MSM in Asia. AIDS **2010**; 24:S30–40.
8. Annual surveillance report of HIV/AIDS in Japan, 1997. AIDS Surveillance Committee, Ministry of Health and Welfare, Japan. Working Group of Annual AIDS Surveillance, Ministry of Health and Welfare, Japan. Jpn J Infect Dis **1999**; 52:55–87.
9. Tsai JJ, Sun HY, Ke LY, et al. Higher seroprevalence of *Entamoeba histolytica* infection is associated with human immunodeficiency virus type 1 infection in Taiwan. Am J Trop Med Hyg **2006**; 74:1016–9.
10. Ohnishi K, Kato Y, Imamura A, Fukayama M, et al. Present characteristics of symptomatic *Entamoeba histolytica* infection in the big cities of Japan. Epidemiol Infect **2004**; 132:57–60.
11. Park WB, Choe PG, Jo JH, et al. Amebic liver abscess in HIV-infected patients, Republic of Korea. Emerg Infect Dis **2007**; 13:516–7.
12. Hung CC, Chen PJ, HsiE. histolytica SM, et al. Invasive amoebiasis: an emerging parasitic disease in patients infected with HIV in an area endemic for amoebic infection. AIDS **1999**; 13:2421–8.
13. Hung CC, Deng HY, Hsiao WH, et al. Invasive amebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. Arch Intern Med **2005**; 165:409–15.
14. Hung CC, Ji DD, Sun HY, et al. Increased risk for *Entamoeba histolytica* infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. PLoS Negl Trop Dis **2008**; 2:e175. doi:10.1371/journal.pntd.0000175.
15. Samie A, Barrett LJ, Bessong PO, et al. Seroprevalence of *Entamoeba histolytica* in the context of HIV and AIDS: the case of Vhembe district, in South Africa's Limpopo province. Ann Trop Med Parasitol **2010**; 104:55–63.
16. Stauffer W, Abd-Alla M, Ravdin JI. Prevalence and incidence of *Entamoeba histolytica* infection in South Africa and Egypt. Arch Med Res **2006**; 37:266–9.
17. del Carmen Sanchez-Guillen M, Velazpuez-Rojas M, Salgado-Rosas H, et al. Seroprevalence of anti-*Entamoeba histolytica* antibodies by IHA and ELISA assays in blood donors from Puebla, Mexico. Arch Med Res **2000**; 31:S53–4.
18. Cross JH, Tsai SH. Indirect hemagglutination antibody titers for *Entamoeba histolytica* in dried filter paper blood and sera. Southeast Asian J Trop Med Public Health **1982**; 13:69–72.
19. Chacin-Bonilla L, Mathews H, Dikdan Y, Guanipa N. Seroepidemiologic study of amebiasis in a community of the State of Zulia, Venezuela. Rev Inst Med Trop Sao Paulo **1990**; 32:467–73.
20. Caballero-Salcedo A, Viveros-Rogel M, Salvatierra B, et al. Seroepidemiology of amebiasis in Mexico. Am J Trop Med Hyg **1994**; 50:412–9.
21. Uga S, Ono K, Kataoka N, Hasan H. Seroepidemiology of five major zoonotic parasite infections in inhabitants of Sidoarjo, East Java, Indonesia. Southeast Asian J Trop Med Public Health **1996**; 27:556–61.
22. Yang B, Chen Y, Wu L, Wu L, Tachibana H, Cheng X. Seroprevalence of *Entamoeba histolytica* infection in China. Am J Trop Med Hyg **2012**; 87:97–103.
23. Hung CC, Wu PY, Chang SY, et al. Amebiasis among persons who sought voluntary counseling and testing for human immunodeficiency virus infection: a case-control study. Am J Trop Med Hyg **2011**; 84:65–9.
24. Chang SY, Sun HY, Ji DD, et al. Cost-effectiveness of detection of intestinal amebiasis by using serology and specific-amebic-antigen assays among persons with or without human immunodeficiency virus infection. J Clin Microbiol **2008**; 46:3077–9.
25. Takeuchi T, Miyahira Y, Kobayashi S, Nozaki T, Motta SR, Matsuda J. High seropositivity for *Entamoeba histolytica* infection in Japanese homosexual men: further evidence for the occurrence of pathogenic strains. Trans R Soc Trop Med Hyg **1990**; 84:250–1.
26. Takeuchi T, Okuzawa E, Nozaki T, et al. High seropositivity of Japanese homosexual men for amebic infection. J Infect Dis **1989**; 159:808.

27. Watanabe K, Gatanaga H, Escueta-de C, Tanuma J, Nozaki T, Oka S. Amebiasis in HIV-1-infected Japanese men: clinical features and response to therapy. PLoS Negl Trop Dis **2011**; 5:e1318. doi:10.1371/journal.pntd.0001318.
28. Nagata N, Shimbo T, Akiyama J, et al. Risk factors for intestinal invasive amebiasis in Japan, 2003–2009. Emerg Infect Dis **2012**; 18:717–24.
29. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev **2007**; 20:511–32.
30. Garcia LS, Bruckner DA, Brewer TC, Shimizu RY. Comparison of indirect fluorescent-antibody amoebic serology with counterimmunoelectrophoresis and indirect hemagglutination amoebic serologies. J Clin Microbiol **1982**; 15:603–5.
31. Haque R, Duggal P, Ali IM, et al. Innate and acquired resistance to amebiasis in Bangladeshi children. J Infect Dis **2002**; 186:547–52.
32. Jackson TF, Anderson CB, Simjee AE. Serological differentiation between past and present infection in hepatic amoebiasis. Trans R Soc Trop Med Hyg **1984**; 78:342–5.

Cancer Letters 342 (2014) 52–59

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

HIV protease inhibitor Lopinavir induces apoptosis of primary effusion lymphoma cells via suppression of NF-κB pathway

Ryusho Kariya [a], Manabu Taura [a], Shinya Suzu [a], Hirofumi Kai [b], Harutaka Katano [c], Seiji Okada [a,*]

[a] Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, 2-2-1, Honjo, Kumamoto 860-0811, Japan
[b] Department of Molecular Medicine, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-Honmachi, Kumamoto 862-0973, Japan
[c] Department of Pathology, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjuku, Tokyo 162-8640, Japan

ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form 1 August 2013
Accepted 19 August 2013

Keywords:
Primary effusion lymphoma
HIV-1 protease inhibiton
NF-κB
IκB kinase

ABSTRACT

Primary effusion lymphoma (PEL) is a non-Hodgkin lymphoma that occurs predominantly in patients with advanced AIDS. In this study, we examined the effect of HIV protease inhibitors, Lopinavir (LPV), Ritonavir (RTV) and Darunavir (DRV) on PEL cell lines *in vitro* and *in vivo*. LPV and RTV, but not DRV induced caspase-dependent apoptosis and suppressed NF-κB activity by inhibiting IKK phosphorylation in PEL cells. In a PEL xenograft mouse model, LPV significantly inhibited the growth and invasion of PEL cells. These results suggest that LPV may have promise for the treatment and prevention of PEL, which occurs in HIV/AIDS patients.

1. Introduction

Primary effusion lymphoma (PEL) is a subtype of non-Hodgkin B cell lymphoma that mainly presents in patients with advanced AIDS, but is sometimes also found in immunosuppressed patients, such as those who have undergo organ transplantation [1,2]. Among AIDS-related NHLs, PEL generally has an extremely aggressive clinical course with a median survival of only 6 months [2,3]. PEL usually presents as a lymphomatous effusion in body cavities and is caused by Kaposi sarcoma-associated herpes virus (KSVH/HHV-8) [1]. A number of constitutively activated signaling pathways play critical roles in the survival and growth of PEL cells. These include nuclear factor (NF)-κB, JAK/STAT and PI3 kinase [4–6]. KSHV/HHV-8 encodes a virus Fas-associated death domain-like interleukin-1β-converting enzyme (FLICE) inhibitory protein (vFLIP) that has the ability to activate the NF-κB pathway [7–9]. vFLIP has been shown to bind to the IKK complex to induce constitutive kinase activation [10] and, as a result, PEL cells have high levels of nuclear NF-κB activity, whereas inhibition of NF-κB induces apoptosis in PEL cells [5,11]. These studies support the idea that vFLIP-mediated NF-κB activation is necessary for the survival of PEL cells and that this pathway represents a target for molecular therapy for this disease.

HIV-1 protease inhibitors (HIV-PIs) have been successfully used in the treatment of HIV-1 infection. Incorporation of HIV-PIs in combination antiretroviral therapy (cART) has significantly reduced morbidity and mortality and prolonged the lifespan of patients with HIV infection. However, HIV-PIs have been shown to directly affect cell metabolism, interfere with host proteases and induce metabolic abnormalities such as insulin resistance, lipodystrophy, and hyperlipidemia, even though they were designed to selectively interfere with the catalytic site of HIV protease. Recently, HIV-PIs have become a focus of attention for having anti-tumor effects [12]. HIV-PIs have been shown to block angiogenesis, tumor cell invasion and tumor cell growth, and to induce endothelial reticulum stress, autophagy and tumor cell apoptosis both *in vivo* and *in vitro* [13–15]. Interestingly, the mechanisms of these anti-tumor effects are different with each HIV-PI, indicating that, although classified together, HIV-PIs are quite distinct compounds [16].

Ritonavir (RTV) has been shown to inhibit the chymotrypsin-like activity of the 20S proteasome and to activate the chymotrypsin-like activity of the 26S proteasome conversely [17–19]. RTV also has been reported to inhibit the transactivation of NF-κB induced by activators such as TNFα, HIV-1 Tat protein and the human herpesvirus 8 protein ORF74 [20]. It is possible that inhibition of NF-κB activation by RTV is linked to additional pathways other than proteasome inhibition. HIV-PIs also have been shown to have direct antiangiogenic and antitumor activity [12]. Recently, it was reported that RTV inhibits the growth and infiltration of ATL cells through targeting NF-κB [14,21]. Lopinavir (LPV) is a frequently used HIV-PI, but only a few antitumor effects have been reported [22]. Recently, a second generation HIV-PI,

* Corresponding author. Tel.: +81 96 373 6522; fax: +81 96 373 6523.
E-mail address: okadas@kumamoto-u.ac.jp (S. Okada).

0304-3835/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2013.08.045

Darunavir (DRV), was developed. DRV has high activity against HIV protease and, as a result, DRV does not lead to tolerance induction [23–25], and since DRV is designed to specifically bind to HIV protease, the adverse effects are less frequent compared with other HIV-PIs.

In the present study, we investigated the antitumor activity of HIV-PIs against human PEL cell lines *in vitro* and *in vivo*. We found that RTV and LPV inhibit constitutively active NF-κB, leading to PEL apoptosis. In contrast, a second generation HIV-PI, DRV, has little effect against PEL cells. Our findings provide the experimental basis for utilizing HIV-PIs against tumors in HIV-1-infected individuals.

2. Materials and methods

2.1. Cell lines and reagents

The human PEL cell lines, BCBL-1 (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) [26], BC-1 [27], BC-3 [28], (obtained through ATCC International Essentials of Life Science Research, USA) and TY-1 [29] were maintained in RPMI1640 supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) in a humidified incubator at 37 °C and 5% CO_2. Ritonavir (RTV), Lopinavir (LPV) (Abbott Labs, North Chicago, IL), Darnavir (DRV) (Pfizer, NY) and LY294002 (Cell Signaling Technology, Danvers, MA) were dissolved in DMSO. DHMEQ (a kind gift from Dr. K. Umezawa, Keio University, Japan) is a NF-κB inhibitor that acts at the level of the nuclear translocation of NF-κB [30].

2.2. Tetrazolium dye methylthiotetrazole (MTT) assay

The antiproliferative effects of HIV-PIs against PEL cell lines were measured by the methylthiotetrazole (MTT) method (Sigma, St. Louis, MO). Briefly, 2×10^4 cells were incubated in triplicate in a 96-well microculture plate in the presence of different concentrations of HIV-PIs in a final volume of 0.1 ml for 24 h at 37 °C. Subsequently, MTT (0.5 mg/ml final concentration) was added to each well. After 3 h of additional incubation, 100 μl of a solution of 0.04 N HCl were added to dissolve the crystal. The absorption values at 570 nm were determined with an automatic enzyme-linked immunosorbent assay (ELISA) plate reader (Multiskan, Thermo ElectronVantaa, Finland). Values are normalized to the untreated (control) samples.

2.3. Cell cycle analysis

For cell cycle analysis, after PEL cells were treated with HIV-PIs (25 μM) for various hours, the cells were incubated in 70% ethanol at 4 °C overnight, treated with RNase A and stained with propidium iodide (PI, 50 μg/ml). The DNA content in each cell was analyzed on LSR II flow cytometer (BD Bioscience, San Jose, CA). Data were analyzed on FlowJo software (Tree Star, San Carlos, CA).

2.4. Annexin V assay

Apoptosis was quantified using the Annexin V: FITC apoptosis MEL MEBCYTO apoptosis kit (MBL, Nagoya, Japan) [31]. Briefly, after treatment with various concentration of HIV-PIs for 12 h, cells were harvested, washed with Annexin binding buffer and then incubated with Annexin V-FITC for 15 min in the dark, and PI added before being analyzed on a LSR II cytometer.

2.5. Caspase activity measurements with flow cytometry

Active caspase 3, 8 and 9 activities were measured using APOPCYTO (MBL) according to the manufacturer's instructions. Briefly, various concentrations of HIV-PI- treated or -untreated cells (200 μl) were incubated with 2 μL substrate, FITC-DEVD-FMK was added to each well, and incubated for 60 min at 37 °C in CO_2 incubator. After incubation, cells were washed with washing buffer, 2 μg/ml PI added and analyzed by LSR II. Data were analyzed on FlowJo software for the expression of active caspase 3-, 8- and 9-positive cellular events among PI-negative (living) cells.

2.6. Western blot analysis

BCBL-1 cells with or without treatment of 40 μM LPV for 1, 3 and 6 h were collected and washed in cold PBS before the addition of 300 μl cold buffer A (10 mM HEPES KOH pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.1% NP-40, 0.5 mM DTT, 0.5 mM PMSF, 2 μg/ml pepstatin A, 2 μg/ml aprotinin and 2 μg/ml leupeptin). After incubation on ice for 10 min, the samples were vortexed for 10 s and centrifuged at 5000 rpm for 1 min and supernatant collected as a cytoplasmic sample. Nuclei were pelleted by centrifugation and washed once with buffer A. Then, 100 μl buffer C (50 mM HEPES-KOH pH 7.9, 10% glycerol, 420 mM NaCl, 5 mM MgCl2, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 2 μg/ml pepstatin A, 2 μg/ml aprotinin and 2 μg/ml leupeptin) were added to the nuclei, sonicated for 10 s 10 times and incubated on ice for 3 h. Nuclear extracts were obtained by centrifugation at 15,000 rpm for 15 min. Then the cytoplasmic protein and nuclear extracts (40 μg protein) were separated by 10% SDS–PAGE and blotted onto a PVDF membrane (GE Healthcare, Buckinghamshire, UK). Detection was performed using the ECL Plus Western Blotting Detection System (ECL; GE Healthcare). Primary antibodies used were as follows: anti-p65 (F-6), anti-I-κBα (C-21), anti-IKKαβ (H-479), anti-phospho-IKKαβ (Thr23), anti-actin (C-2), anti-γ tubulin (C-20) (Santa Cruz Biotechnology, Santa Cruz, CA), and and anti-Akt (4691), anti-phospho (Thr308)-Akt (2965), anti phospho (Ser473)-Akt (4060), anti-phospho (Ser241)-PDK1 (3438), anti-PI3 kinase (4257), anti-phospho p85 (Tyr458)/ p55 (Tyr199)-PI3 kinase (4228), anti-phospho-I-κBα (Ser32/36) (Cell Signaling Technology, Danvers, MA). Western blots were quantified using the ImageQuant LAS 4000 system (GE Healthcare). Relative density was evaluated and normalized with actin or γ tubulin.

2.7. Transient transfection and NF-κB p65 reporter gene assay

BCBL-1 was transfected with the NF-κB-LUC plasmid (Stratagene, La Jolla, CA) using the Neon transfection system (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions and incubated at 37 °C for 24 h. Cells were treated with RTV, LPV, DRV and DHMEQ for 1, 3 and 6 h, and protein was extracted and subjected to determination of luciferase activity by the Dual Luciferase Assay system (TOYO INK Corporation, Tokyo, Japan) according to the manufacturer's instructions. Firefly luciferase activity was standardized using luciferase activity by Renilla luciferase activity.

2.8. RT-PCR

Total RNA was extracted from the cells using Trizol (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized from RNA using a PrimeScript RT-PCR kit (Takara Bio, Otsu, Japan) with random primers. The PCR products were analyzed by 1.5% agarose gel electrophoresis and ethidiumbromide staining. Primer sequences were as follows: ORFK13 (v-FLIP): 5′-ATTGACATTAGGGCATCC-3′ and 5′-AAAGGAGGA GGGCAGGTT, ORF72 (v-cyclin): 5′-GATAATAGAGGCGGGCAATG-3′ and 5′-TAA AGCAGGTGTCCAAAGAA-3′, ORF73 (LANA): 5′-GAAGTGGATTACC CTGTTGT TAGC-3′ and 5′-TTGGATCTCGTCTTCCATCC-3′, ORF50 (RTA): 5′-GCC CTCTGC CTTTTGGTT- 3′ and 5′-GATGATGCTGACGGGTGTG-3′, GAPDH: 5′-CGGGAAG CTTGTGATCA ATGG-3′ and 5′-GGCAGTGATGGCATG GACTG-3′[32,33]

2.9. Xenograft mouse model

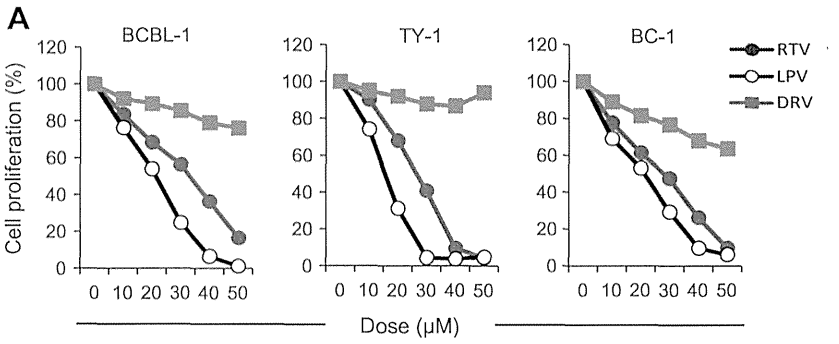
NOD/Scid/Jak3-deficient (NOJ) mice were established by backcrossing Jak3-deficient mice [34] with the NOD.Cg.-Prkdcscid strain for 10 generations [35]. NOJ male mice of 8 to 10 weeks old were housed and monitored in our animal research facility according to the institutional guidelines. All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee at Kumamoto University. NOJ mice were intraperitoneally inoculated with 1×10^7 BCBL-1 cells suspended in 100 μl PBS. The mice were then treated with intraperitoneal injections of PBS or HIV-PIs (40 μmol/kg per day). Tumor burdens were evaluated by measuring the volume of ascites.

2.10. Immunohistochemistry

To investigate the expression of KSHV/HHV-8 ORF73 (LANA) protein, tissue samples were fixed with 10% neutral-buffered formalin, embedded in paraffin and cut into 4 μm sections. The sections were deparaffinized by sequential immersion in xylene and ethanol and rehydrated in distilled water. They were then irradiated for 15 min in a microwave oven for antigen retrieval. Endogenous peroxidase activity was blocked by immersing the sections in methanol/0.6% H_2O_2 for 30 min at room temperature. Affinity-purified PA1-73N antibody [20], diluted 1:3,000 in PBS/5% bovine serum albumin (BSA), was then applied, and the sections were incubated overnight at 4 °C. After washing in PBS twice, the second and third reactions and the amplification procedure were performed using kits according to the manufacturer's instructions (catalyzed signal amplification system kit; DAKO, Copenhagen, Denmark). The signal was visualized using 0.2 mg/ml diaminobenzidine and 0.015% H_2O_2 in 0.05 mol/l Tris–HCl, pH 7.6.

2.11. Statistical analysis

All assays were performed at least in triplicate and expressed as mean values ± SD. The statistical significance of the differences observed between experimental groups was determined using Student's t test. $P < 0.05$ was considered significant.



B

CC50	BCBL-1	TY-1	BC-1	BC-3
RTV	35μM	27μM	28μM	32μM
LPV	22μM	16μM	21μM	20μM
DRV	>50μM	>50μM	>50μM	>50μM

Fig. 1. HIV protease inhibitors inhibit the proliferation of PEL cells. (A) PEL cell lines (BCBL-1, TY-1 BC-1 and BC-3) were incubated with 10, 20, 30, 40, 50 μM RTV, LPV DRV for 24 h. A cell proliferation assay was carried out using MTT as described in Section 2. A representative result from 3 independent experiments is shown. (B) Show the IC50 value of each HIV protease inhibitors.

3. Results

3.1. RTV and LPV cause dose-dependent inhibition of the proliferation and apoptosis of PEL cell lines

We initially sought to determine whether HIV-PI treatment leads to the inhibition of PEL cell proliferation. Four PEL cell lines (BCBL-1, TY-1, BC-1 and BC-3) were cultured in the presence of 10, 20, 30, 40, and 50 μM HIV-PIs for 24 h, and proliferation was analyzed by MTT assays. Fig. 1A shows that as the dose of RTV and LPV increased from 10 to 50 μM, cell growth inhibition increased in a dose-dependent fashion in all PEL cell lines (Fig. 1A). On the other hand, marked anti-proliferative effects were not observed by DRV at these concentrations. Fig. 1B shows the IC50 value of each HIV-PI individual. In subsequent experiments, we determined whether the observed suppressive effects of RTV and LPV in the MTT assay were due to the induction of cell cycle arrest or apoptosis. As shown in Fig. 2, 25 μM RTV and LPV treatment for 12 h induced cell cycle arrest. Next we used Annexin V and propidium iodide dual staining to detect apoptosis. Annexin-positive propidium iodide-negative fraction represents the early phase of apoptosis whereas Annexin-positive propidium iodide-positive fraction represents the late phase of apoptosis and necrosis [31]. As shown in Fig. 3A, 40 μM RTV and LPV treatment for 12 h caused apoptosis in all cell lines tested, but DRV did not induce apoptosis in these cell lines. Next, we measured the activation of caspase 3, 8 and 9 to further confirm that RTV and LPV induced apoptosis in PEL cells. As shown in Fig. 3B, RTV and LPV treatment of PEL cells induced the activation of caspase 3, 8 and 9, a hallmark of cells undergoing apoptosis.

3.2. LPV efficiently blocks the constitutive NF-κB activity of PEL cell lines

As several reports have suggested that NF-κB can act as a survival factor and is required for the proliferation of PEL cells, and PEL cells are known to induce apoptosis with inhibition of the NF-κB pathway, [5,11,36] we examined whether LPV inhibits NF-κB activation. When PEL cell lines were treated with 40 μM LPV for 1, 3 and 6hr, the amount of phosphorylated I-κBα protein was severely reduced; however, the amount of I-κBα protein was almost the same, indicating that LPV suppresses NF-κB activity by suppressing the activation of I-κBα phosphorylation (Fig. 4A). Suppression of I-κB phosphorylation blocked the nuclear translocation of NF-κB p65 and led to the accumulation of NF-κB p65 protein (Fig. 4A). Next, we fractioned nuclear protein and analyzed the expression of p65 by Western blotting (Fig. 4B) to confirm NF-κB p65 suppression by LPV. When PEL cell lines were treated with 40 μM LPV for 6 h, the amount of nuclear NF-κB p65 protein was reduced as expected, indicating that LPV suppresses NF-κB activity. To confirm that LPV could inhibit NF-κB transcriptional activity in PEL cell lines, we performed the NF-κB promoter assay. Treatment with RTV and LPV suppressed the transcriptional activity of NF-κB in all cell lines tested (Fig. 4C); however, DRV did not inhibit NF-κB activity. These results revealed that LPV blocks the constitutive NF-κB activity of PEL cells.

3.3. Inhibition of NF-κB activity is not depend on Akt pathway

Akt pathway regulates NF-κB activity and it was reported that LPV inhibits Akt pathway[37]. Therefore, we examined whether LPV suppresses IKK phosphorylation through inhibition of Akt pathway. When PEL cell lines were treated with 40 μM LPV for 1, 3 and 6 h, the amount of phosphorylated Akt protein was severely reduced. However amount of phosphorylated PI3 kinase which is upstream of Akt pathway was almost same indicated that LPV suppressed Akt phosphorylation (Fig. 5A) Furthermore, we examined whether inhibition of Akt pathway suppresses IKK phosphorylation in PEL cells. LY294002 (PI3 kinase inhibitor) treatment suppressed Akt phosphorylation but could not suppressed IKK phosphorylation (Fig. 5B), indicated that inhibition of Akt pathway cannot suppress IKK phosphorylation in PEL cells. These results indicate that suppression of IKK phosphorylation by LPV is not due to the inhibition of Akt pathway.

3.4. LPV dose not induce HHV-8 reactivation

It is well known that v-FLIP has the ability to activate the NF-κB pathway through binding to the IKK complex to induce

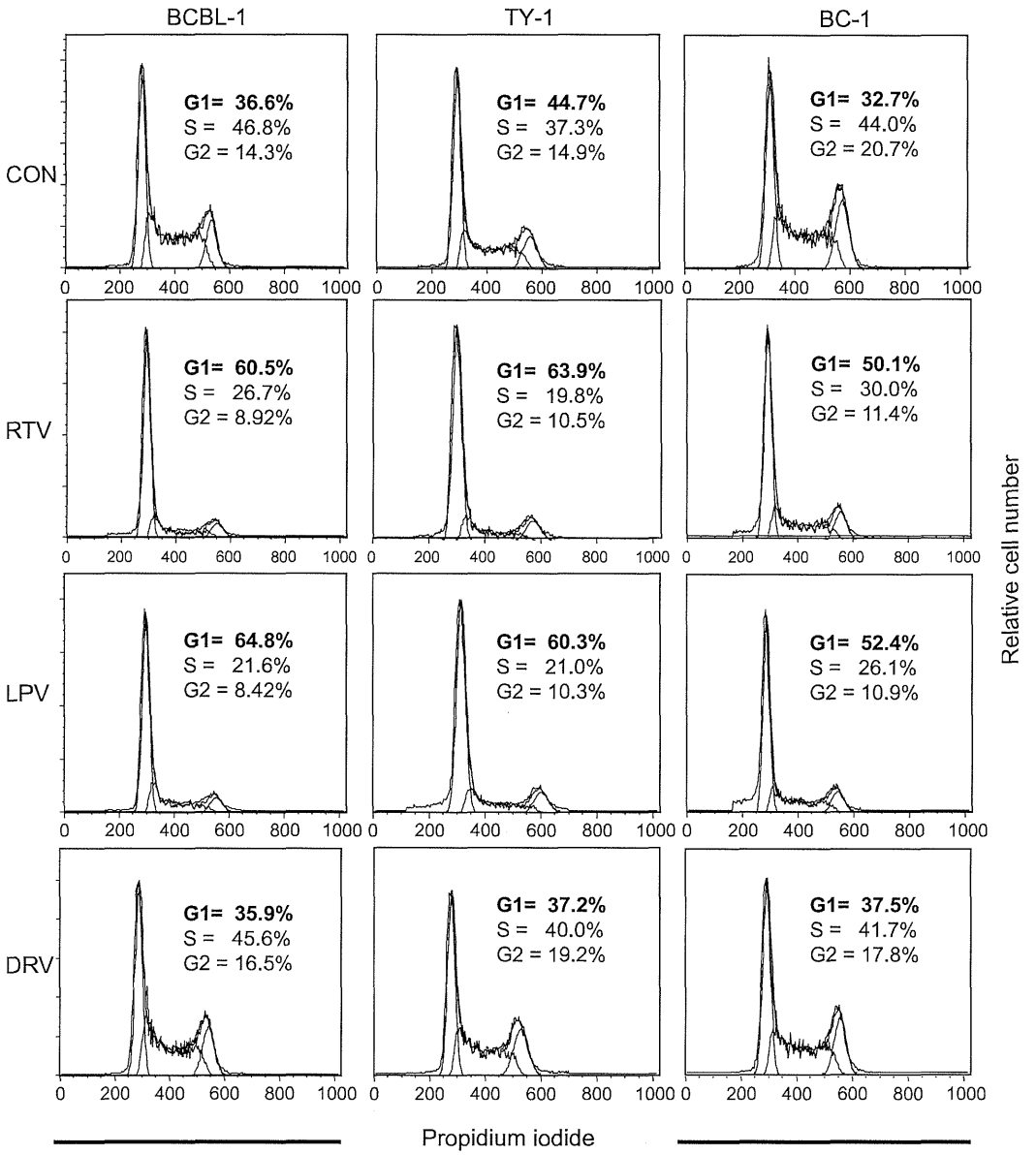


Fig. 2. HIV protease inhibitors cause cell cycle arrest of PEL cells. PEL cell lines BCBL-1, TY-1 and BC-1 were treated with RTV, LPV, DRV (25 μM) for 12 h, and DNA histograms were determined and the cell cycle was analyzed using FlowJo software. A representative result from 3 independent experiments is shown.

constitutive kinase activation [7,36,38]. Therefore, RT-PCR was performed to examine the effects of LPV on viral gene expression including v-FLIP. LPV treatment did not change both of lytic (RTA) and latent gene (v-FLIP, LANA and v-cyclin) expression (Fig. 5C), indicated that LPV have no direct effect on viral gene expression including v-FLIP.

3.5. Antitumor effect of LPV in PEL-inoculated mice

As the above results suggested the efficacy of RTV and LPV for the treatment of PEL patients, we next examined the *in vivo* effects of LPV in a PEL-inoculated mouse model. Severely immunodeficient, NOD/Scid/Jak3-deficient mice (NOJ mice) [35] were inoculated intraperitoneally with 1×10^7 BCBL-1 cells. BCBL-1 produced massive ascites within 4 weeks of inoculation (Fig. 6A). As PEL is characterized by lymphomatous effusion of serous cavities and rarely presents with a definable tumor mass [1,2], these mice are a clinically relevant PEL model. A dose of 40 μmol/kg/day LPV and DRV or PBS was administrated via intraperitoneal injection on day 3 after cell inoculation and every day thereafter for 28 days. LPV-treated mice appeared to be healthy and had a significantly lower volume of ascites than DRV or PBS-treated mouse ascites (0.47 ± 0.35 ml vs. 5.08 ± 1.52 ml, $n = 10$ each, $p < 0.001$) (Fig. 6B). Organ infiltration by tumor cells was analyzed and evaluated by hematoxylin–eosin staining and LANA immunostaining (Fig. 6C). We found that mice inoculated intraperitoneally with BCBL-1 exhibited infiltration into the lung, liver, and spleen without macroscopic lymphoma formation. The number of LANA-positive cells in LPV-treated mice was significantly reduced (0–1 cells per field magnification, 340) compared to non-treated, DRV mouse (10–20) cells per field magnification, 340) (Fig. 6D). These data indicate that LPV significantly inhibits the growth and infiltration of PEL cells *in vivo*.

4. Discussion

In the present study, we investigated the effects of three different HIV PIs, RTV, LPV and DRV, on PEL cells both *in vitro* and *in vivo*.

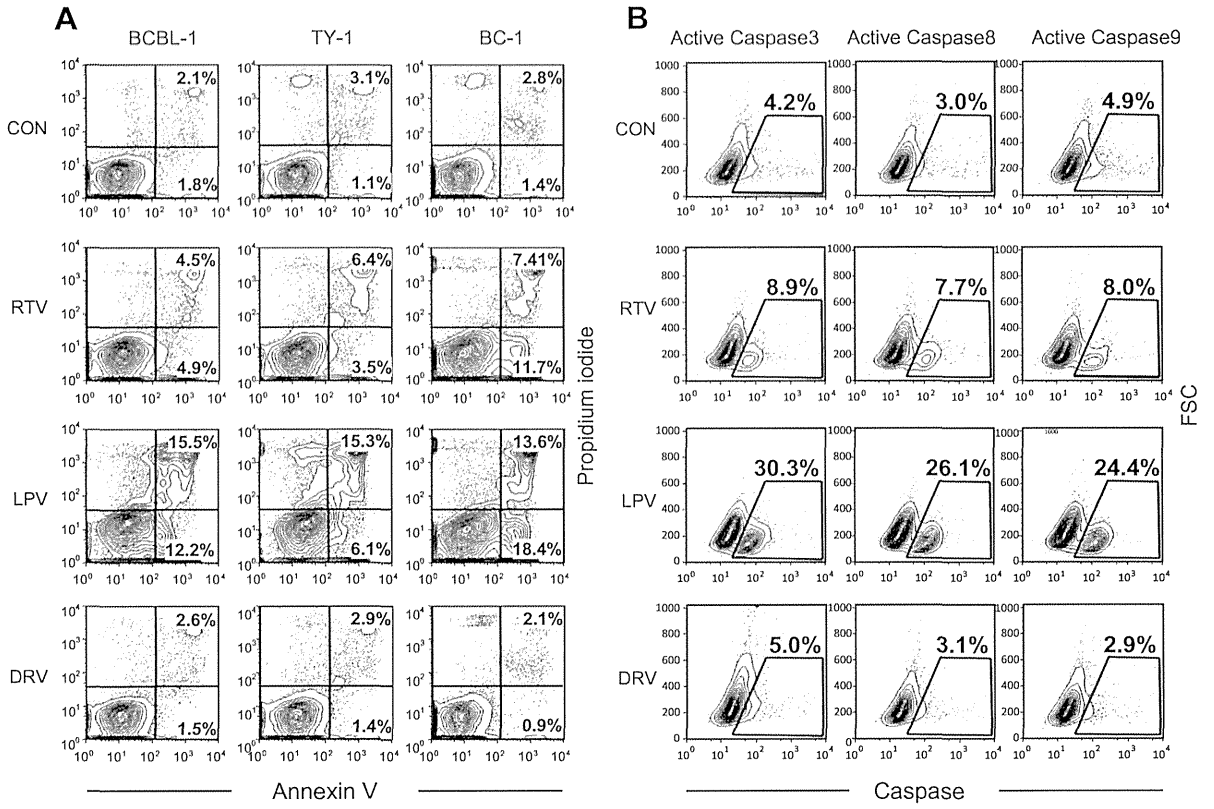


Fig. 3. HIV protease inhibitors cause apoptosis of PEL cells. (A) HIV protease inhibitors induced apoptosis as detected by Annexin V and propidium iodide dual staining. PEL cell lines BCBL-1, TY-1 and BC-1 were treated with 40 μM of RTV, LPV and DRV for 12 h and were subsequently stained with Annexin-FITC and propidium iodide before being analyzed by flow cytometry. (B) HIV protease inhibitor induces apoptosis of PEL cells via caspase-3-, 8- and 9-dependent pathway. The PEL cell line BCBL-1 was treated with RTV LPV DRV (40 μM) for 12 h and was subsequently stained with caspase-3, 8, 9 before being analyzed by flow cytometry. A representative result from 3 independent experiments is shown.

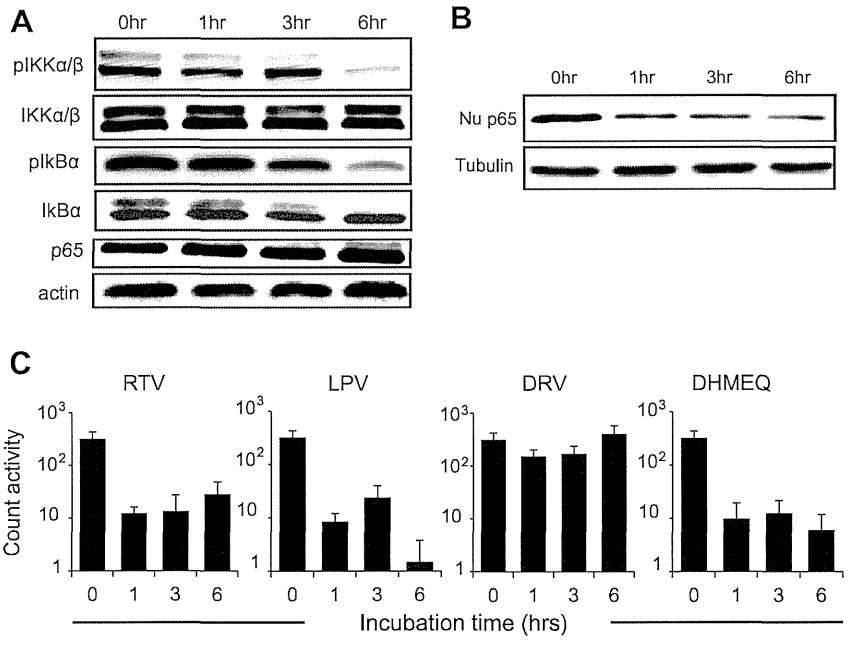


Fig. 4. Inhibitory effects of HIV protease inhibitors on the expression of NF-κB pathways. (A) The PEL cell line BCBL-1 was treated with LPV (40 μM) for 1, 3 and 6hr and cytoplasmic proteins were extracted and Western blot was performed. The numbers indicate the relative expression of each protein level normalized with actin. (B) The PEL cell line BCBL-1 was treated with LPV (40 μM) for 1, 3 and 6 h and nuclear proteins were extracted and Western blot was performed to detect NF-κB p65. The numbers indicate the relative expression of p65 normalized with γ tubulin. A representative result from 3 independent experiments is shown. (C) BCBL-1 was transfected with NF-κB-LUC plasmid and 24 h later cells were treated with 40 μM of RTV, LPV, DRV and DHMEQ for 1, 3, 6 h and protein was extracted, and firefly and Renilla luciferase were measured on a luminometer. Firefly luciferase activity was normalized to Renilla luciferase expression.

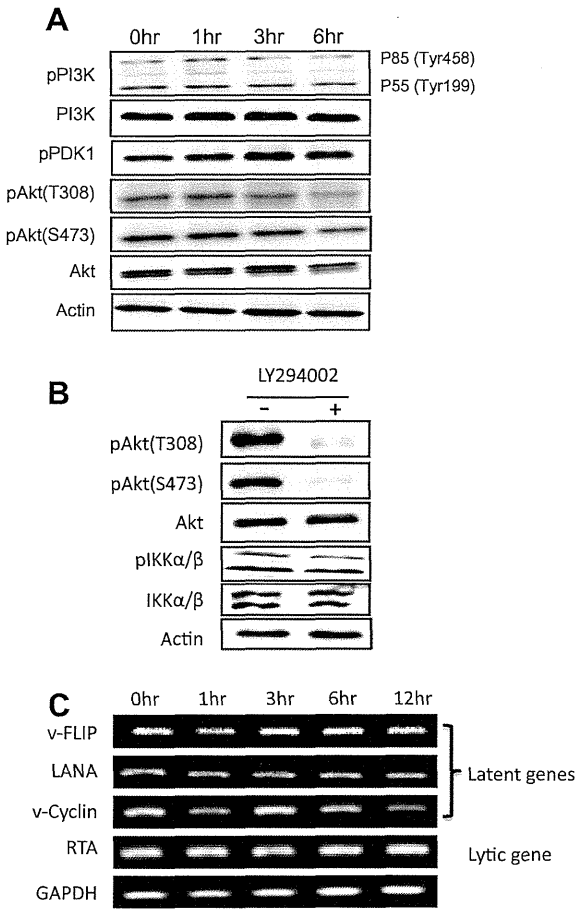


Fig. 5. Effects of LPV against upstream of IKKα and IKKβ. (A) The PEL cell line BCBL-1 was treated with LPV (40 μM) for 1, 3 and 6hr and cytoplasmic proteins were extracted and Western blot was performed. (B) The PEL cell line BCBL-1 was treated with LY294002 (50 μM) for 3hr and cytoplasmic proteins were extracted and Western blot was performed. (C) Viral gene expression after treatment with LPV (40 μM) for 1, 3 and 6hr was examined by RT-PCR.

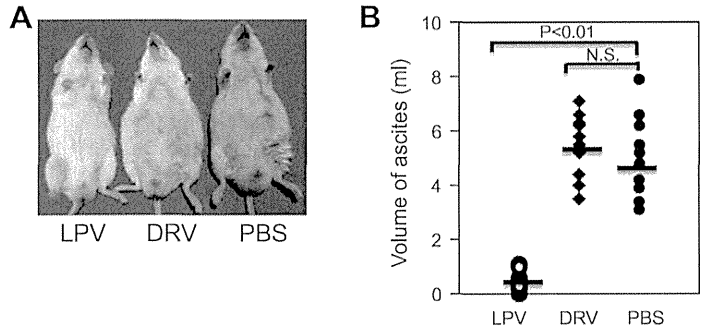
Our results showed that RTV and LPV but not DRV exhibited potent pro-apoptotic effects on PEL cells and provided evidence that such apoptosis occurs via then inhibition of NF-κB activity. These direct anti-tumor effects were shown in animal models free of viruses and immunocompetent cells such as T cells and NK cells, and in which direct anti-tumor effects of HIV-PIs that were independent of drug-mediated HIV suppression and immune reconstitution could be detected.

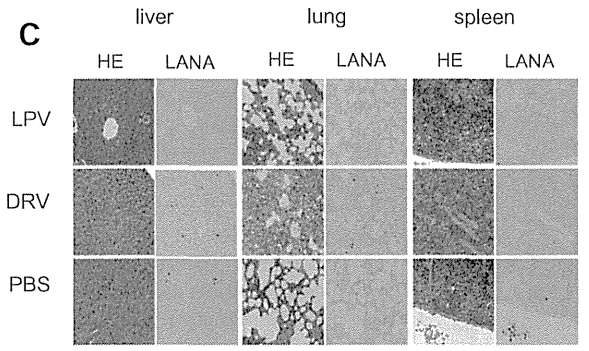
PEL is an incurable, aggressive B-cell malignancy and most patients that suffer from it respond poorly to traditional chemotherapy and develop chemoresistance. A novel strategy for treatment is needed for this aggressive lymphoma. Several agents have been tested in the search for a more effective treatment for PEL. It is now postulated that the mechanisms of lymphomagenesis involve the deregulation of several signaling pathways that may act either independently or crosstalk with each other. These include NF-κB, JAK/STAT and PI3 kinase pathways [4–6] in the case of PEL. PEL is associated with KSHV/HHV-8 infection and KSHV/HHV-8 contains a homologue of the cellular FLIP protein vFLIP, which has the ability to activate the NF-κB pathway through binding to the IKK complex to induce constitutive kinase activation. [7,36,38] Moreover, inhibition of NF-κB activity leads to the apoptosis of KSHV-infected PEL cells [5,11]. These results suggest that the NF-κB pathway is an effective target for the treatment of PEL. Activation of NF-κB is involved in various kinds of cancer development and progression [39–41] as well as in virus-associated lymphomas, indicating that NF-κB is a good molecular target for cancer treatment. HIV-PIs, such as RTV, LPV and DRV, have been successfully used in clinical treatments of HIV infection, with patients exhibiting a marked decrease in HIV viral load and a subsequent increase in CD4+ T-Cell counts [42–45]. Antitumor effects of HIV-PIs are expected, since patients treated with HAART but failing in CD4 T cell recovery still show a significantly lower risk of AIDS-related malignancies [46,47]. In fact, antitumor effects and the induction of apoptosis by HIV-PIs have been reported in the last 10 years [14,19,21]. In our study, we demonstrated that RTV and LPV are able to suppress the growth of PEL cells and induce apoptosis via the inhibition of NF-κB activity, especially by blocking the phosphorylation of I-κBα (Fig. 4A).

It was previously reported that HIV-PIs inhibit proteasome activity [21,48], and proteasome inhibition leads to the accumulation of I-κBα resulting in inhibition of NF-κB pathway. However, we could not detect any I-κBα protein accumulation in our study (Fig. 4A), indicating that LPV induced NF-κB inhibition is not due to the proteasome inhibition. In the present study, we showed that LPV inhibited IKKα and IKKβ phosphorylation (Fig. 4A). IKKα and IKKβ have been pursued by many groups as targets for the development of therapeutic agents to be used for the treatment of cancer, as well as inflammatory and metabolic diseases [49]. Akt and TAK1 have been identified as upstream kinase for IKKα and IKKβ and it was already reported that several HIV-PIs including LPV inhibit Akt pathway [37]. In present study, we also detected that LPV suppressed Akt pathway (Fig. 5A). However, specific inhibitor of Akt phosphorylation (LY294002) could not suppress IKKα and IKKβ phosphorylation in PEL line (Fig. 5B). It was also reported that TAK1 specific inhibitor cannot inhibit v-FLIP induced IKK kinase activation [50]. Taken together, Akt and TAK1 are not important molecule for v-FLIP mediated IKK phosphorylation in PEL cells. Actually, v-FLIP has the ability to activate NF-κB pathway thorough the direct binding to IKKγ [7,36,38]. If LPV induced HHV-8 lytic activation, v-FLIP expression is reduced and suppress NF-κB pathway. However, lytic activation of HHV-8 was not observed with the addition of LPV(Fig. 5C). v-FLIP is not a kinase that activates the IKK complex by inducing its phosphorylation. Instead, v-FLIP activates the complex by direct interaction via a mechanism believed to involve a conformational change of IKK complex [51]. Our findings emphasize the implication of v-FLIP for PEL survival and LPV might inhibit IKK phosphorylation by blocking of the v-FLIP and IKKγ binding at least independent of Akt.

Although designed to target only the HIV protease, HIV protease inhibitors are known to cause toxicity in patients, such as insulin resistance and lipodystrophy, suggesting that HIV protease inhibitors have other targets in mammalian cells. In fact, one of the most potent HIV-PIs, Nelfinavir, exerted pleiotropic biochemical and cellular effects on cancer cells that included the induction of endoplasmic reticulum (ER) stress, autophagy, and apoptosis *in vitro* and *in vivo* [52]. These preclinical studies suggested the idea that HIV-PIs can reduce the risk of cancer among HIV-1-infected persons. As HIV-1-infected patients have a higher risk of cancers than the uninfected population, and the complication of cancer is becoming one of the most important life-threatening events, if HIV-PIs can reduce the risk of cancer, this is a benefit for HIV-1-infected patients. Until now, only one cohort study has been performed and the risk of cancer was the same among those using HIV-PI or HIV-PI-sparing regimens [53]. Additional studies using large registries and post-marketing surveillance are needed to provide further data on the possible relationship between HIV-PIs and cancer inhibition among HIV-infected persons.

DRV was designed to form robust interactions with the protease enzyme from many strains of HIV-1, including strains from patients with multiple resistance mutations to HIV-PIs. DRV did not show these antitumor effects or inhibitory effects on the NF-κB





D

No.	drug	spleen	liver	lung
4096	LPV	-	-	+/-
4097	LPV	-	-	-
4098	DRV	++	++	++
4099	DRV	++	++	++
4100	PBS	+	+	+
4101	PBS	++	+	+

++: 1 area(x100)>10 points
+: 1 area (x100) 1-9 points
-: no points

Fig. 6. Treatment of NOD/Scid/Jak3-deficient mice with HIV protease inhibitor LPV suppresses the development and metastasis of KSHV-associated lymphoma *in vivo*. (A) A photograph of LPV, DRV-treated and non-treated ascites-bearing mice 4 weeks after being inoculated with BCBL-1 intraperitoneally. (B) Quantization of volume of ascites. (C) Hematoxylin-eosin staining and immunohistochemical staining using anti-LANA (PA1–73 N antibody) was performed to detect BCBL-1 in liver, lungs and spleen. (D) Quantification of BCBL-1 metastasis.

pathway in our study. DRV has very high activity against HIV-1 protease [23–25], but not against mammalian proteases, indicating the lack of anti-tumor effects as well as few adverse effects. In fact, clinical studies have demonstrated that DRV has more potent antiviral efficacy with more favorable lipid profiles that other HIV-PIs[54]. Thus, appropriate and creative use of HIV-PIs is required based on their actions and adverse effects.

In conclusion, we have shown the ability of HIV-PI, RTV and LPV to induce cell death through blocking the NF-κB pathway in PEL cells. Our study provides a rationale for a clinical trial of HIV-PIs in patients with PEL and other NF-κB-activated tumors. Further investigations aimed at determining the efficacy of HIV-PIs are warranted and may lead to the development of new effective therapies for this intractable lymphoma.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

We thank Ms. I. Suzu for technical assistance and Ms. K. Tokunaga for secretarial assistance. This work was supported in part by Health and Labour Sciences Research Grants from the Ministry of Health, Labour, and Welfare of Japan (H22-AIDS-I-002 and H25-AIDS-I-002) and by the Global COE program "Global Education and Research Center Aiming at the Control of AIDS", and Grants-in-Aid for Science Research (Nos. 23107725, 25114711, and 25460499) from the Ministry of Education, Science, Sports, and Culture of Japan.

References

[1] R.G. Nador, E. Cesarman, A. Chadburn, D.B. Dawson, M.Q. Ansari, J. Sald, D.M. Knowles, Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus, Blood 88 (1996) 645–656.
[2] Y.B. Chen, A. Rahemtullah, E. Hochberg, Primary effusion lymphoma, Oncologist 12 (2007) 569–576.
[3] E. Cesarman, Y. Chang, P.S. Moore, J.W. Said, D.M. Knowles, Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas, New Engl. J. Med. 332 (1995) 1186–1191.
[4] Y. Aoki, G.M. Feldman, G. Tosato, Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma, Blood 101 (2003) 1535–1542.
[5] S.A. Keller, E.J. Schattner, E. Cesarman, Inhibition of NF-kappaB induces apoptosis of KSHV-infected primary effusion lymphoma cells, Blood 96 (2000) 2537–2542.
[6] S. Uddin, A.R. Hussain, K.A. Al-Hussein, P.S. Manogaran, A. Wickrema, M.I. Gutierrez, K.G. Bhatia, Inhibition of phosphatidylinositol 3′-kinase/AKT signaling promotes apoptosis of primary effusion lymphoma cells, Clin. Cancer Res. 11 (2005) 3102–3108.
[7] Q. Sun, H. Matta, P.M. Chaudhary, The human herpes virus 8-encoded viral FLICE inhibitory protein protects against growth factor withdrawal-induced apoptosis via NF-kappa B activation, Blood 101 (2003) 1956–1961.

[8] A. Jarviluoma, P.M. Ojala, Cell signaling pathways engaged by KSHV, Biochim. Biophys. Acta 1766 (2006) 140–158.
[9] P.J. Jost, J. Ruland, Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications, Blood 109 (2007) 2700–2707.
[10] L. Liu, M.T. Eby, N. Rathore, S.K. Sinha, A. Kumar, P.M. Chaudhary, The human herpes virus 8-encoded viral FLICE inhibitory protein physically associates with and persistently activates the Ikappa B kinase complex, J. Biol. Chem. 277 (2002) 13745–13751.
[11] S.A. Keller, D. Hernandez-Hopkins, J. Vider, V. Ponomarev, E. Hyjek, E.J. Schattner, E. Cesarman, NF-kappaB is essential for the progression of KSHV- and EBV-infected lymphomas in vivo, Blood 107 (2006) 3295–3302.
[12] P. Monini, C. Sgadari, E. Toschi, G. Barillari, B. Ensoli, Antitumour effects of antiretroviral therapy, Nat. Rev. Cancer 4 (2004) 861–875.
[13] O. Touzet, A. Philips, Resveratrol protects against protease inhibitor-induced reactive oxygen species production, reticulum stress and lipid raft perturbation, AIDS 24 (2010) 1437–1447.
[14] C. Sgadari, G. Barillari, E. Toschi, D. Carlei, I. Bacigalupo, S. Baccarini, C. Palladino, P. Leone, R. Bugarini, L. Malavasi, A. Cafaro, M. Falchi, D. Valdembri, G. Rezza, F. Bussolino, P. Monini, B. Ensoli, HIV protease inhibitors are potent anti-angiogenic molecules and promote regression of Kaposi sarcoma, Nat. Med. 8 (2002) 225–232.
[15] E. Toschi, C. Sgadari, L. Malavasi, I. Bacigalupo, C. Chiozzini, D. Carlei, D. Compagnoni, S. Bellino, R. Bugarini, M. Falchi, C. Palladino, P. Leone, G. Barillari, P. Monini, B. Ensoli, Human immunodeficiency virus protease inhibitors reduce the growth of human tumors via a proteasome-independent block of angiogenesis and matrix metalloproteinases, Int. J. Cancer 128 (2011) 82–93.
[16] W.A. Chow, C. Jiang, M. Guan, Anti-HIV drugs for cancer therapeutics: back to the future?, Lancet Oncol 10 (2009) 61–71.
[17] P. Andre, M. Groettrup, P. Klenerman, R. de Giuli, B.L. Booth Jr., V. Cerundolo, M. Bonneville, F. Jotereau, R.M. Zinkernagel, V. Lotteau, An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation, and T cell responses, Proc. Natl. Acad. Sci. USA 95 (1998) 13120–13124.
[18] G. Schmidtke, H.G. Holzhutter, M. Bogyo, N. Kairies, M. Groll, R. de Giuli, S. Emch, M. Groettrup, How an inhibitor of the HIV-I protease modulates proteasome activity, J. Biol. Chem. 274 (1999) 35734–35740.
[19] S. Gaedicke, E. Firat-Geier, O. Constantiniu, M. Lucchiari-Hartz, M. Freudenberg, C. Galanos, G. Niedermann, Antitumor effect of the human immunodeficiency virus protease inhibitor ritonavir: induction of tumor-cell apoptosis associated with perturbation of proteasomal proteolysis, Cancer Res. 62 (2002) 6901–6908.
[20] H. Katano, Y. Sato, T. Kurata, S. Mori, T. Sata, High expression of HHV-8-encoded ORF73 protein in spindle-shaped cells of Kaposi's sarcoma, Am. J. Pathol. 155 (1999) 47–52.
[21] S. Pati, C.B. Pelser, J. Dufraine, J.L. Bryant, M.S. Reitz Jr., F.F. Weichold, Antitumorigenic effects of HIV protease inhibitor ritonavir: inhibition of Kaposi sarcoma, Blood 99 (2002) 3771–3779.
[22] M.D. Johnson, M. O'Connell, W. Pilcher, Lopinavir inhibits meningioma cell proliferation by Akt independent mechanism, J. Neurooncol. 101 (2011) 441–448.
[23] N.M. King, M. Prabu-Jeyabalan, E.A. Nalivaika, P. Wigerinck, M.P. de Bethune, C.A. Schiffer, Structural and thermodynamic basis for the binding of TMC114, a next-generation human immunodeficiency virus type 1 protease inhibitor, J. Virol. 78 (2004) 12012–12021.
[24] S. De Meyer, H. Azijn, D. Surleraux, D. Jochmans, A. Tahri, R. Pauwels, P. Wigerinck, M.P. de Bethune, TMC114, a novel human immunodeficiency virus type 1 protease inhibitor active against protease inhibitor-resistant viruses, including a broad range of clinical isolates, Antimicrob. Agents Chemother. 49 (2005) 2314–2321.
[25] R. Ortiz, E. Dejesus, H. Khanlou, E. Voronin, J. van Lunzen, J. Andrade-Villanueva, J. Fourie, S. De Meyer, M. De Pauw, E. Lefebvre, T. Vangeneugden, S. Spinosa-Guzman, Efficacy and safety of once-daily darunavir/ritonavir versus Lopinavir/ritonavir in treatment-naive HIV-1-infected patients at week 48, Aids 22 (2008) 1389–1397.
[26] R. Renne, W. Zhong, B. Herndier, M. McGrath, N. Abbey, D. Kedes, D. Ganem, Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture, Nat. Med. 2 (1996) 342–346.
[27] E. Cesarman, P.S. Moore, P.H. Rao, G. Inghirami, D.M. Knowles, Y. Chang, In vitro establishment and characterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi's sarcoma-associated herpesvirus-like (KSHV) DNA sequences, Blood 86 (1995) 2708–2714.
[28] L. Arvanitakis, E.A. Mesri, R.G. Nador, J.W. Said, A.S. Asch, D.M. Knowles, E. Cesarman, Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus, Blood 88 (1996) 2648–2654.
[29] H. Katano, Y. Hoshino, Y. Morishita, T. Nakamura, H. Satoh, A. Iwamoto, B. Herndier, S. Mori, Establishing and characterizing a CD30-positive cell line harboring HHV-8 from a primary effusion lymphoma, J. Med. Virol. 58 (1999) 394–401.
[30] K. Umezawa, A. Ariga, N. Matsumoto, Naturally occurring and synthetic inhibitors of NF-kappaB functions, Anticancer Drug Des. 15 (2000) 239–244.
[31] I. Vermes, C. Haanen, H. Steffens-Nakken, C. Reutelingsperger, A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V, J. Immunol. Methods 184 (1995) 39–51.
[32] H.H. Krishnan, P.P. Naranatt, M.S. Smith, L. Zeng, C. Bloomer, B. Chandran, Concurrent expression of latent and a limited number of lytic genes with immune modulation and antiapoptotic function by Kaposi's sarcoma-associated herpesvirus early during infection of primary endothelial and fibroblast cells and subsequent decline of lytic gene expression, J. Virol. 78 (2004) 3601–3620.
[33] S. Shimasaki, T. Koga, T. Shuto, M.A. Suico, T. Sato, K. Watanabe, S. Morino-Koga, M. Taura, S. Okada, K. Mori, H. Kai, Endoplasmic reticulum stress increases the expression and function of toll-like receptor-2 in epithelial cells, Biochem. Biophys. Res. Commun. 402 (2010) 235–240.
[34] S.Y. Park, K. Saijo, T. Takahashi, M. Osawa, H. Arase, N. Hirayama, K. Miyake, H. Nakauchi, T. Shirasawa, T. Saito, Developmental defects of lymphoid cells in Jak3 kinase-deficient mice, Immunity 3 (1995) 771–782.
[35] S. Okada, H. Harada, T. Ito, T. Saito, S. Suzu, Early development of human hematopoietic and acquired immune systems in new born NOD/Scid/Jak3null mice intrahepatic engrafted with cord blood-derived CD34+ cells, Int. J. Hematol. 88 (2008) 476–482.
[36] I. Guasparri, S.A. Keller, E. Cesarman, KSHV vFLIP is essential for the survival of infected lymphoma cells, J. Exp. Med. 199 (2004) 993–1003.
[37] M. Kraus, J. Bader, H. Overkleeft, C. Driessen, Nelfinavir augments proteasome inhibition by bortezomib in myeloma cells and overcomes bortezomib and carfilzomib resistance, Blood Cancer J. 3 (2013) e103.
[38] Q. Sun, S. Zachariah, P.M. Chaudhary, The human herpes virus 8-encoded viral FLICE-inhibitory protein induces cellular transformation via NF-kappaB activation, J. Biol. Chem. 278 (2003) 52437–52445.
[39] M. Karin, F.R. Greten, NF-kappaB: linking inflammation and immunity to cancer development and progression, Nat. Rev. Immunol. 5 (2005) 749–759.
[40] J. Inoue, J. Gohda, T. Akiyama, K. Semba, NF-kappaB activation in development and progression of cancer, Cancer Sci. 98 (2007) 268–274.
[41] H.J. Kim, N. Hawke, A.S. Baldwin, NF-kappaB and IKK as therapeutic targets in cancer, Cell Death Differ. 13 (2006) 738–747.
[42] A.C. Collier, Efficacy of combination antiretroviral therapy, Adv. Exp. Med. Biol. 394 (1996) 355–372.
[43] A.C. Collier, R.W. Coombs, D.A. Schoenfeld, R. Bassett, A. Baruch, L. Corey, Combination therapy with zidovudine, didanosine and saquinavir, Antiviral Res. 29 (1996) 99.
[44] A.C. Collier, R.W. Coombs, D.A. Schoenfeld, R.L. Bassett, J. Timpone, A. Baruch, M. Jones, K. Facey, C. Whitacre, V.J. McAuliffe, H.M. Friedman, T.C. Merigan, R.C. Reichman, C. Hooper, L. Corey, Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group, New Engl. J. Med. 334 (1996) 1011–1017.
[45] M. Markowitz, M. Saag, W.G. Powderly, A.M. Hurley, A. Hsu, J.M. Valdes, D. Henry, F. Sattler, A. La Marca, J.M. Leonard, et al., A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection, New Engl. J. Med. 333 (1995) 1534–1539.
[46] O. Kirk, C. Pedersen, A. Cozzi-Lepri, F. Antunes, V. Miller, J.M. Gatell, C. Katlama, A. Lazzarin, P. Skinhoj, S.E. Barton, Non-Hodgkin lymphoma in HIV-infected patients in the era of highly active antiretroviral therapy, Blood 98 (2001) 3406–3412.
[47] P. Monini, E. Toschi, C. Sgadari, I. Bacigalupo, C. Palladino, D. Carlei, G. Barillari, B. Ensoli, The use of HAART for biological tumour therapy, J. HIV Ther. 11 (2006) 53–56.
[48] C. Bono, L. Karlin, S. Harel, E. Mouly, S. Labaume, L. Galicier, S. Apcher, H. Sauvageon, J.P. Fermand, J.C. Bories, B. Arnulf, The human immunodeficiency virus-1 protease inhibitor nelfinavir impairs proteasome activity and inhibits the proliferation of multiple myeloma cells in vitro and in vivo, Haematologica 97 (2012) 1101–1109.
[49] M. Karin, Mitogen activated protein kinases as targets for development of novel anti-inflammatory drugs, Ann. Rheum. Dis. 63 (Suppl. 2) (2004) ii62–ii64.
[50] H. Matta, R. Gopalakrishnan, C. Graham, B. Tolani, A. Khanna, H. Yi, Y. Suo, P.M. Chaudhary, Kaposi's sarcoma associated herpesvirus encoded viral FLICE inhibitory protein K13 activates NF-kappaB pathway independent of TRAF6, TAK1 and LUBAC, PLoS ONE 7 (2012) e36601.
[51] C. Bagneris, A.V. Ageichik, N. Cronin, B. Wallace, M. Collins, C. Boshoff, G. Waksman, T. Barrett, Crystal structure of a vFlip-IKKgamma complex: insights into viral activation of the IKK signalosome, Mol. Cell 30 (2008) 620–631.
[52] J.J. Gills, J. Lopiccolo, J. Tsurutani, R.H. Shoemaker, C.J. Best, M.S. Abu-Asab, J. Borojerdi, N.A. Warfel, E.R. Gardner, M. Danish, M.C. Hollander, S. Kawabata, M. Tsokos, W.D. Figg, P.S. Steeg, P.A. Dennis, Nelfinavir, A lead HIV protease inhibitor, is a broad-spectrum, anticancer agent that induces endoplasmic reticulum stress, autophagy, and apoptosis in vitro and in vivo, Clin. Cancer Res. 13 (2007) 5183–5194.
[53] N.F. Crum-Cianflone, K.H. Hullsiek, V. Marconi, A. Weintrob, A. Ganesan, R.V. Barthel, S. Fraser, M.P. Roediger, B. Agan, S. Wegner, The impact of nelfinavir exposure on cancer development among a large cohort of HIV-infected patients, J. Acquir. Immune Defic. Syndr. 51 (2009) 305–309.
[54] J.S. Currier, C. Martorell, O. Osiyemi, M.T. Yin, R. Ryan, G. De La Rosa, J. Mrus, Effects of darunavir/ritonavir-based therapy on metabolic and anthropometric parameters in women and men over 48 weeks, AIDS Patient Care STDS 25 (2011) 333–340.

 The Journal of AIDS Research

活動報告

活用状況を考慮した「拠点病院診療案内」のあり方についての検討
——拠点病院診療案内の活用に関するアンケート調査結果より——

Investigation of Usefulness of AIDS Core Hospital List
——Findings of Questionnaire Survey about the Utilization of AIDS Core Hospital List——

須貝　恵[1,8]，鈴木　智子[2,8]，センテノ田村恵子[3,8]，辻　典子[4,8]，
井内亜紀子[5,8]，濱本　京子[6,8]，吉用　緑[7,8]，山本　政弘[7]

Megumi SUGAI[1,8], *Tomoko SUZUKI*[2,8], *Keiko CENTENOTAMURA*[3,8], *Noriko TSUJI*[4,8], *Akiko IUCHI*[5,8], *Kyoko HAMAMOTO*[6,8], *Midori YOSHIMOCHI*[7,8] *and Masahiro YAMAMOTO*[7]

[1] 新潟大学医歯学総合病院感染管理部，[2] 国立病院機構仙台医療センター，[3] 北海道大学病院，
[4] 石川県立中央病院，[5] 国立病院機構大阪医療センター，[6] 広島大学病院，
[7] 国立病院機構九州医療センター，[8] 公益財団法人エイズ予防財団リサーチレジデント

[1] Niigata University Medical and Dental Hospital, [2] National Hospital Organization Sendai Medical Center, [3] Hokkaido University Hospital, [4] Ishikawa Prefectural Central Hospital, [5] National Hospital Organization Osaka National Hospital, [6] Hiroshima University Hospital, [7] National Hospital Organization Kyushu Medical Center, [8] Japan Foundation for AIDS Prevention

はじめに

厚生労働省エイズ対策研究事業「HIV感染症の医療体制の整備に関する研究」班では，冊子「拠点病院診療案内」（以下，「診療案内」）を全国のエイズ治療拠点病院（以下，拠点病院）の協力により，2001年度より作成し，関係機関に配布している。「診療案内」は全国拠点病院のHIV/エイズ診療に関する情報を中心に病院情報を掲載し，拠点病院や保健所等の施設間連携の促進を目的として，ブロック拠点病院情報担当職員を中心に毎年編纂を行い，拠点病院の最新情報を提供してきた。今回，今後の「診療案内」，ならびにそのWebサイト化に向けて情報内容を充実・向上させることを目的として「拠点病院診療案内の活用に関するアンケート」を行った。

対象および方法

2010年5月から8月に「診療案内2009-2010」を配布した1,127施設（全国拠点病院378施設，行政機関747施設，その他2施設）のHIV診療や関連業務に関わるスタッフを対象とした。「拠点病院診療案内の活用に関するアンケート」調査票を送付し，FAXで回答を得た。

著者連絡先：須貝　恵（〒951-8520　新潟市中央区旭町通1-754　新潟大学医歯学総合病院感染管理部）

2013年2月12日受付：2013年5月16日受理

結　果

622施設から934件の回答を得た（回答率55%）。回答者は行政関係者59%，病院関係者41%と行政関係者の回答率が高かった。回答者職種比率は保健師が36%，医師22%，看護師11%で，薬剤師6%，技師6%，ソーシャルワーカー5%，事務4%，心理職3%であった。

「診療案内」の利用有無は，「ある」が全体で50%であり，機関別では行政関係が52%，病院関係が50%であった。

利用用途は，最も多かったのが行政関係では「患者・HIV検査受検者への情報提供」，病院関係では「患者転居にともなう拠点病院検索のため」であった（図1）。

利用目的は達せられたかの問いには，「十分」が77%，「必要な情報の掲載が不十分であったが目的は達した」が21%で，98%が目的は達したと回答した。「必要な情報の掲載が不十分で目的は果たせなかった」が2%あった。

掲載が不十分であったとされた必要な情報は，「新しい情報」，「担当課，担当医師（記載が無い）」，「具体的な受診手続き」，「実際の診療実績」，「出産可能かどうか」，「地域の情報」，「土曜日，夜間の受診について」，「通訳の有無」などであった。

「診療案内」に施設情報を掲載するにあたり，担当者の明確化など，院内で確認したことがあったかの問いでは，「全ての掲載事項は掲載依頼前に決まっていた」219件（72%），「一部の掲載事項は掲載依頼前に決まっていたが，

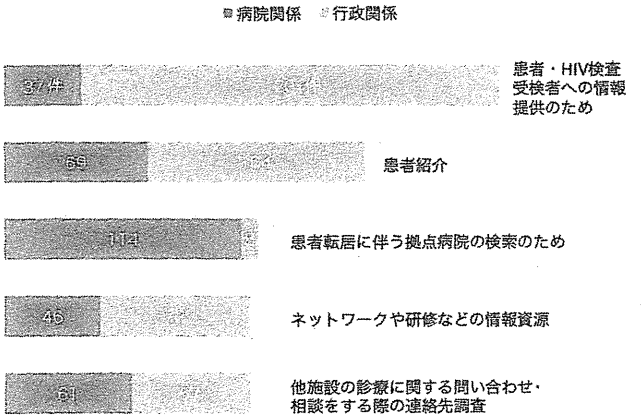


図 1 診療案内をどのようなことに利用したか

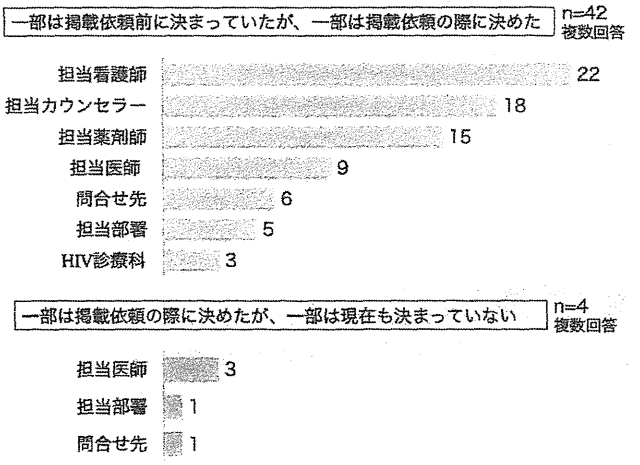


図 2 掲載依頼の際に決定した項目

一部は掲載依頼の際に担当者を決めた」42 件（14%），「一部の掲載事項は掲載依頼前に決まっていたが，一部は現在も決まっていない」24 件（8%），「全ての掲載事項は掲載依頼の際に決めた」16 件（5%），「一部の掲載事項は掲載依頼の際に決めたが，一部は現在も決まっていない」4 件（1%）であった。

掲載依頼の際に，「全て」および「一部」の担当者を決めたという回答は 20%であった。掲載の際に決定した事項は，「一部は掲載依頼前に決まっていたが，一部は掲載依頼の際に決めた」群では，看護師，薬剤師，カウンセラーを決めたという回答が多かった。「一部は掲載依頼の際に決めたが，一部は現在も決まっていない」群は，担当医師，担当部署，問合せ先という回答であった（図 2）。

調査結果から活かしたこと

アンケート結果をもとに掲載内容の改訂を行った。まず 2011-2012 年版では，HIV 医療に欠かせない社会資源利用に係わる「指定自立支援医療機関の有無」，「身体障害者福祉法 15 条第一項の指定医師の存在」，「MSW の担当者欄」を追加し，地図を詳細なものに変更した。保健所等から回答が多かった「初診時予約の要・不要」についても追加した。さらに 2012-2013 年版では，患者の HIV 診療担当科以外のさまざまな診療科への受診の必要性から，「診療科別の診療実績」を追加した。

2011 年には「拠点病院診療案内 WEB」を立ち上げた（http : //hiv-hospital.jp/）。

今後の課題

今回のアンケート結果から不十分とされた「新しい情報」，「担当課，担当医師（記載が無い）」，「具体的な受診手続き」，「実際の診療実績」，「出産可能かどうか」，「地域の情報」，「土曜日，夜間の受診について」，「通訳の有無」といった内容の充実が必要である。

最新の情報の更新については冊子が年 1 回の発行であることを考えると限界があるため，情報の修正が容易な Web サイトで随時情報を更新することで最新情報の提供というニーズを満たすことが可能になると考える。

ま と め

「診療案内」は，患者・HIV 検査受検者への病院情報の提供や紹介先・連携先の拠点病院を検索する情報源として活用されていたが，まだまだ不十分な点が多いことが明らかとなった。一方で「掲載依頼の際に担当者を決めた」とする回答が 20%あり，掲載依頼により担当者の明確化が促され，「診療案内」が HIV 診療体制整備の一助となっていると考えられた。今後も各職種の担当者掲載を続けることで，担当者の明確化が継続的に行われ，自施設の診療体制の振り返りや整備，拠点病院としての職員の意識向上へとつながっていくことが望まれる。

これからも，冊子・Web サイトそれぞれの特徴を生かし，効率的な情報収集および提供体制，その運用について検討していきたい。

謝辞

本研究は，厚生労働科学研究費「HIV 感染症の医療体制の整備に関する研究」の一環として行った。「拠点病院診療案内」作成および，アンケートにご協力いただいた全国拠点病院，その他関係各所の皆さまに深謝致します。

活動報告

拠点病院の患者紹介現状から考える医療体制の課題
——拠点病院から拠点病院以外の医療機関への患者紹介実績調査結果より——

Problems of the Medical System about the Patient Referral of AIDS Core Hospitals
——Investigation of Patient Referral of AIDS Core Hospitals to Medical Institutions Other than AIDS Core Hospitals——

須貝　恵[1,8]，辻　典子[2,8]，吉用　緑[3,8]，センテノ田村恵子[4,8]，
鈴木　智子[5,8]，井内亜紀子[6,8]，濱本　京子[7,8]，山本　政弘[3]

Megumi SUGAI[1,8], *Noriko TSUJI*[2,8], *Midori YOSHIMOCHI*[3,8], *Keiko CENTENOTAMURA*[4,8], *Tomoko SUZUKI*[5,8], *Akiko IUCHI*[6,8], *Kyoko HAMAMOTO*[7,8] *and Masahiro YAMAMOTO*[3]

[1] 新潟大学医歯学総合病院感染管理部，[2] 石川県立中央病院，[3] 国立病院機構九州医療センター，
[4] 北海道大学病院，[5] 国立病院機構仙台医療センター，[6] 国立病院機構大阪医療センター，
[7] 広島大学病院，[8] 公益財団法人エイズ予防財団リサーチレジデント

[1] Niigata University Medical and Dental Hospital, [2] Ishikawa Prefectural Central Hospital, [3] National Hospital Organization Kyushu Medical Center, [4] Hokkaido University Hospital, [5] National Hospital Organization Sendai Medical Center, [6] National Hospital Organization Osaka National Hospital, [7] Hiroshima University Hospital, [8] Japan Foundation for AIDS Prevention

はじめに

HIV 感染症が抗 HIV 薬による治療継続により管理可能な疾患となった現在，大都市圏での患者集中の問題のみならず，地方においても患者の高齢化，ならびにさまざまな合併症を伴う患者が増加している。その結果，拠点病院間はもとより，拠点病院以外の医療機関との連携の必要性が増している。

厚生労働省エイズ対策研究事業「HIV 感染症の医療体制の整備に関する研究」班では，全国拠点病院の HIV/エイズ診療に関する情報を中心に病院情報を掲載した冊子，「拠点病院診療案内」（以下，「診療案内」）を作成し，拠点病院や保健所等における施設間連携の促進を目的として拠点病院の最新情報を提供してきた。

「診療案内」はブロック拠点病院情報担当職員を中心に毎年編纂を続けてきたが，2010 年にわれわれは「拠点病院診療案内の活用に関するアンケート調査」を実施し「診療案内」に対するニーズを検討し，その結果をもとに掲載内容の改訂，「拠点病院診療案内 WEB」のたちあげ等を行った。

著者連絡先：須貝　恵（〒951-8520　新潟市中央区旭町通 1-754　新潟大学医歯学総合病院感染管理部）

2013 年 2 月 12 日受付；2013 年 4 月 19 日受理

さらに 2011 年には，「診療案内」の内容充実と今後の HIV 医療体制構築にいかすために，拠点病院から拠点病院以外の病院や診療所/クリニックへの患者紹介実績を調査したので報告する。

対象および方法

2011 年 5 月から 6 月に「拠点病院診療案内 2010-2011」に掲載の拠点病院 379 施設を対象にアンケートを送付し，郵送にて回答を得た。

結　果

総回答数 228 施設で，回答率 60.2% であった。拠点病院以外の病院への患者紹介は 31%（71 施設）の拠点病院が行っていた。2010 年においては，拠点病院以外の病院への入院患者紹介が 28 施設で，外来患者紹介が 33 施設で行われていた。診療科別でみると，入院では内科 22 件，透析 1 件，精神科 8 施設，歯科 1 施設，その他 5 施設だった。外来では内科 11 施設，透析 2 施設，精神科 9 施設，歯科 21 施設，その他 4 施設だった。診療所/クリニックへの紹介実績は 39 施設で行われていた。内訳は，歯科がいちばん多く 28 施設，内科 16 施設，精神科 10 施設，透析 4 施設，その他診療科（肛門科，皮膚科，整形外科，眼科，外科，泌尿器科，リハビリ，等）9 施設であった（図 1）。

紹介先施設を選んだ理由（複数選択可）は入院では，「紹介先施設が受入れに協力的であった」が16件，「紹介元（拠点病院）からの働きかけにより，受入れに理解・協力を得た」が12件であった。外来では，「紹介先施設が受入れに協力的であった」が22件，「患者さんの生活を考慮し，通院/入院がしやすい場所であった」が19件，「患者さん自身が紹介先を希望した/患者さんの以前からのかかりつけであった」が11件であった。

診療所/クリニックでは「紹介先施設が受入れに協力的であった」が31件，「患者さんの生活を考慮し，通院/入院がしやすい場所であった」が30件，「患者さん自身が紹介先を希望した/患者さんの以前からのかかりつけであった」が23件であった（図2）。

診療科別の紹介先を選んだ理由では，内科は「患者さんの生活を考慮し，通院/入院がしやすい場所であった」がいちばん多い理由であったが，透析，精神科，歯科では，「紹介先施設が受入れに協力的であった」がいちばん多い理由であった。

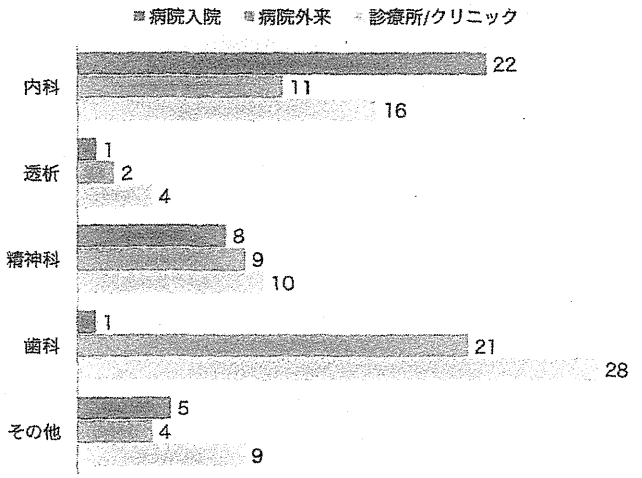


図 1　診療科別にみた紹介実績ありの施設数

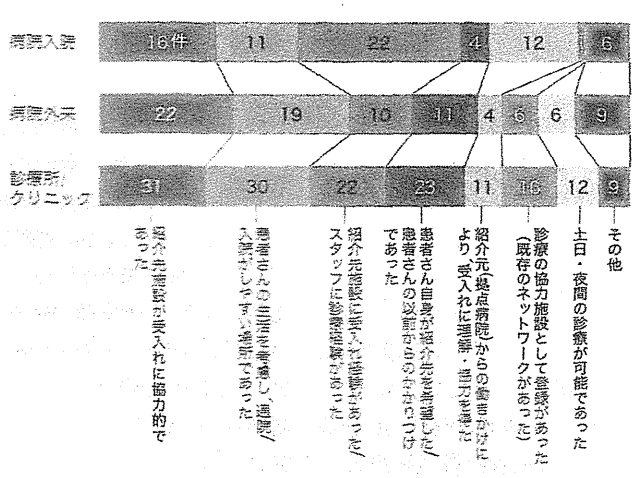


図 2　紹介先を選んだ理由（複数選択可回答）

考　　察

病院への入院紹介は外来紹介・診療所/クリニックに比べ，「紹介元からの働きかけ」との回答が多く，「患者の生活を考慮」「患者の希望，かかりつけ」が少なかった。紹介先（転院先）を選ぶ際は治療上の理由が優先されることが多いからではないかと考えられる。病院外来・診療所/クリニックへの紹介は，病院入院に比べ「患者の生活圏の考慮」や「患者の希望/かかりつけ」との回答が多く，外来通院中で全身状態が比較的良好であることから，医療の面に加えて，より生活状況を重視した転院を担当医も患者の側も考慮することが伺われる。長期コントロールにおいては数カ月に1回程度の受診でHIV感染症診療は対応可能となっている。その反面，透析医療においては週に数回以上の通院が必要であり，歯科治療においても受診継続や早期受診，あるいは緊急受診が必要な場合があり，拠点病院以外でも患者ニーズにあった医療機関の選択が重要である。

歯科は他科に比べて「診療の協力施設として登録があった」と回答する割合が多かった。地域によっては，研究班，行政，歯科医師会などによるネットワーク構築が有効に機能していると考えられる[1]。

紹介先診療科は多岐にわたっており，自施設に該当科がないという理由で緩和ケアや精神科などの診療科への転院がみられた。HIV感染症が長期的な治療継続により管理可能な疾患となったことで，HIV感染症管理以外の生活習慣や加齢に伴う疾患の管理が重要となってきていることから，多岐にわたる診療科受診の必要性は今後ますます多くなると考える。

また，受入医療機関の開拓には拠点病院の働きかけが主体である現状が示された。その働きかけが拠点病院や中核拠点病院だけの負担にならないよう，ブロック拠点病院が必要に応じてバックアップしていく体制を継続していかなければならないと考える。

今後の課題

HIV感染症診療担当科以外のさまざまな診療科への受診の必要性から，「診療案内2012-2013」では全診療科の診療経験の有無の項目を追加した。本調査で示されたように拠点病院に該当診療科がないことで拠点病院以外への紹介も多くみられており，HIV感染を原因として患者の受診施設が制限されることがないよう，医療体制の整備を進めなければならない。歯科の診療ネットワークは徐々に広がってきており[1]，他の診療科でもネットワークの構築は有効であると考える。そのためにも行政や地域医療関係機関と

協働して地域の現状に応じた医療体制を目指し，研修会や情報共有の場を企画するなどの取組みを検討し，HIV診療の理解と協力への働きかけを継続的に行いながら，地域医療機関とのネットワークを構築していく必要がある。

今後はブロック拠点病院，中核拠点病院，拠点病院そして地域医療機関の担うべき役割を理解し，そのうえで現在のHIV診療に見合った医療体制の改善につなげていけるような協力体制を図ることが重要である。

その一助となるべく「診療案内」の掲載情報については，引き続き検討を重ねていきたい。

謝辞

本研究は，厚生労働科学研究費「HIV感染症の医療体制の整備に関する研究」の一環として行った。「拠点病院診療案内」作成および，アンケートにご協力いただいた全国拠点病院，その他関係各所の皆さまに深謝致します。

文　献

1）前田憲昭：歯科のHIV医療体制整備．厚生労働科学研究費補助金エイズ対策研究事業HIV感染症の医療体制の整備に関する研究（研究代表者 山本政弘）．平成23年度総括・分担研究報告書：74-77，2012.

症　例

十二指腸乳頭部腫瘍が疑われたHIV感染合併CMV感染症の1例

国立病院機構九州医療センター内科・臨床研究センター

南　留美　高濱宗一郎　中嶋恵理子　山本　政弘

（平成24年8月31日受付）
（平成25年4月17日受理）

Key words: HIV, cytomegalovirus, duodenitis

序　文

サイトメガロウイルス（CMV）感染症は，エイズ指標疾患の1つであり，免疫能の低下したHIV感染者に比較的よく合併する感染症である．HIV感染者においてCMVは消化管の多様な部位に感染し，特に食道，胃，大腸に頻度が高い．十二指腸病変は頻度が低く[1]検索範囲内でも症例報告が散見される程度である．CMV感染に伴う消化管病変は潰瘍を形成することが多く，出血や穿孔を起こしうる．今回，我々は，十二指腸乳頭部に腫瘤性病変をきたしたために十二指腸乳頭部腫瘍を疑われたHIV感染合併CMV十二指腸炎の症例を経験したので報告する．

症　例

症例：59歳，男性．

主訴：味覚障害，食欲不振，体重減少（－13kg/6カ月）．

既往歴：梅毒，B型・C型肝炎の既往なし．その他特記事項無し．

家族歴：母，糖尿病．

生活歴：性指向は不明．海外渡航歴なし．薬物使用歴なし．喫煙なし．飲酒は付き合い程度．

現病歴：2009年7月頃から，味覚障害，口腔内疼痛が出現し近医受診するも症状は改善しなかった．2009年11月，食欲不振，体重減少を認めたため，近医にて上部消化管内視鏡施行．その際，カンジダ性食道炎および十二指腸乳頭部腫瘍性病変が認められた．HIV抗体スクリーニング検査（EIA法）陽性であったため，2009年12月，当院紹介受診．

初診時現症：体温36.7度，脈拍72/分，整，眼球結膜黄疸なし，眼瞼結膜貧血なし．口腔内白苔あり．頸部リンパ節腫大なし，心音，呼吸音正常．腹部平坦，軟，圧痛なし，腫瘤触知せず．

初診時検査所見：末梢血検査はWBC 4,300/μLと正常，リンパ球16.2%と低下，RBC 435万/μL，Hb 11.2g/dLと軽度の貧血を認めた．生化学検査では肝・胆道系酵素の上昇なし，アミラーゼ上昇なし．空腹時血糖233mg/dL，HbA1c 7.9%（JDS）と耐糖能障害を認めた．CA19-9，CEAの上昇なし．HIV感染に関しては，HIV-1抗体確認検査（ウエスタンブロット法）陽性．血漿中HIV-RNA量26万コピー/mLであった．CD4陽性細胞数49/μLと細胞性免疫の低下を認めた．活動性CMV感染症の指標である血中CMV抗原（C7 HRP）は8/52,000cellsと陽性であった．上部消化管内視鏡（Fig. 1a）では，カンジダ性食道炎および十二指腸乳頭周囲に不整な潰瘍を伴う腫瘤性病変を認め，肉眼上，乳頭部癌を疑う所見であった．腹部造影CTでは，十二指腸乳頭部付近に造影効果のある壁の肥厚を認めるも主膵管や総胆管の拡張は認めなかった．その他の腸管には壁在性の炎症や浸潤を示唆する瀰漫性の腸壁の肥厚や狭窄，腫瘤形成等の病変を認めなかった．

臨床経過（Fig. 2）：十二指腸乳頭部の腫瘤性病変は病理所見上，粘膜の糜爛や線維化を伴い，細胞質内封入体を持つ細胞浸潤を粘膜上皮および間質に認めた（Fig. 3a）．浸潤している細胞は免疫染色にてCMV陽性であったことより（Fig. 3b），CMV性十二指腸乳頭部炎と診断した．眼底所見では，網膜血管周囲に出血を伴う白斑を認めCMV網膜症と診断した．カンジダ食道炎，CMV感染症以外には，明らかな日和見感染症の合併を認めなかった．免疫再構築症候群（Imuune reconstitution inflammatory syndrome；IRIS）によるCMV感染症の増悪を防ぐためvalganciclovir 1,800mg/日にて4週間治療を行い，CMV網膜症に関しては白斑の減少，硝子体への細胞浸潤の低下

別刷請求先：（〒810-8563）福岡市中央区地行浜1—8—1
国立病院機構九州医療センター臨床研究センター　南　留美

Fig. 1 Endoscopic findings of the papilla of Vater
(a) The first examination, (b) 3 weeks after the medication of valganciclovir, (c) 6 months after the initiation of ART.

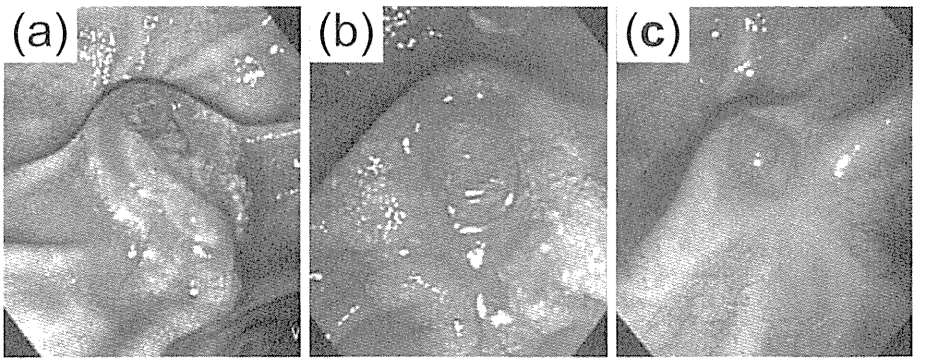
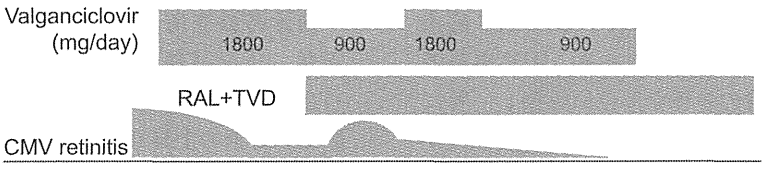


Fig. 2 Clinical course
RAL; raltegravir, TVD; Tenofovir/Emtricitabin



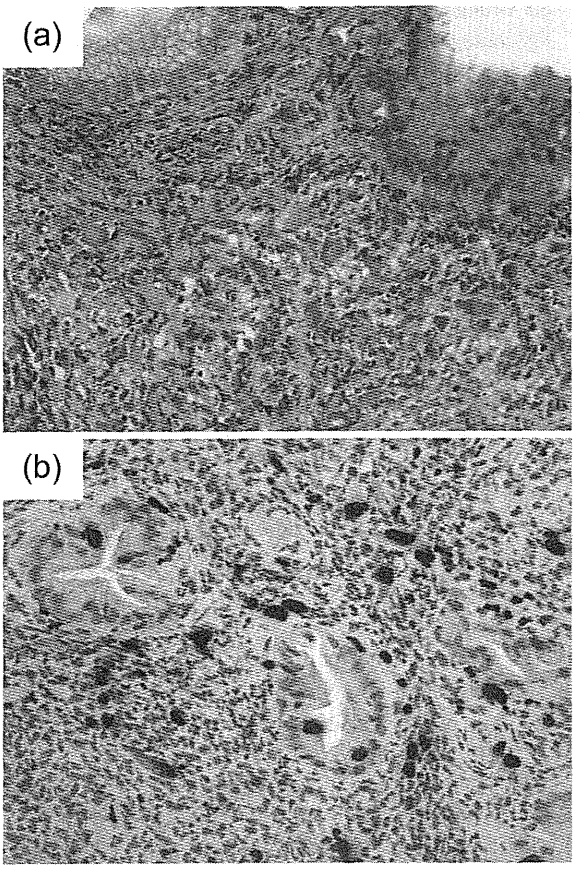
	Jan	Feb	Mar	Apr	May	June	Jul	Aug
CD4 (/μL)	49	52	91	72	104	124	113	168
HIV-RNA (/mL)	260000	110000	160	<40	130	130	200	94
CMV (C7HRP)	8/52000	(-)	(-)	(-)	(-)	(-)	(-)	(-)

を確認，CMV 性十二指腸乳頭部炎に関しては，不整な潰瘍および腫瘤が縮小し軽度の発赤を残す程度にまで改善しているのを確認後（Fig. 1b），valganciclovir を維持量（900mg/日）に減量し，抗 HIV 療法（raltegravir＋tenofovir/emtricitabine）を開始した．抗 HIV 療法開始 1 カ月後に，眼底所見にて硝子体への細胞浸潤の増加を認めた．IRIS による CMV 網膜症の悪化と考え，valganciclovir を一旦治療量（1,800mg/日）に増量し，その後改善した．CMV 性十二指腸乳頭部炎に関しては，IRIS を疑わせる所見は認めなかった．血中 HIV-RNA は，抗 HIV 療法開始後 2 カ月で感度以下（＜40 コピー/mL），CD4 陽性細胞数は抗 HIV 療法開始後 1 年で 200/μL 以上に回復した．初診時に認められた耐糖能異常に関しては，インスリン分泌量の低下を認めなかったことより，遺伝的要因（母親が糖尿病）および HIV 感染に伴うインスリン抵抗性の亢進が原因と考えられ，抗 HIV 剤開始後，食事療法のみで改善した．抗 HIV 薬開始 6 カ月後，十二指腸病変は内視鏡にて消失しており（Fig. 3c），組織学的にも CMV 陽性細胞は検出されなかった．以後，当科外来にて経過を見ているが，十二指腸乳頭部炎，網膜症ともに再燃せず 3 年以上経過している．

考　察

CMV 感染は，免疫不全の進行した HIV 感染症患者においてもっとも頻度の高い日和見感染の 1 つである．AIDS 患者においては約 50% が血中 CMV 抗原陽性であり[2]，剖検では 60～90% に感染が認められたと報告がある[3)4)]．CMV はあらゆる臓器に感染しうるが，消化管は肺，副腎についで感染する頻度が高い臓器である．消化管においては，回腸末端や右側結腸が好発部位である[5]．多発性に病変を認めることもあり，特に，十二指腸乳頭部炎の場合は，胆道系に感染を併発していることが多いと報告されている[6]．本症例の場合，血液生化学所見からも画像所見からも胆道系感染の合併は認められなかった．また，腹部造影 CT 上，大腸病変も認められなかった．本症例のように十二指腸単独に病巣を認めることは稀と報告されており，実際，1990 年以降の MEDLINE による検索では HIV 感染合併の CMV 十二指腸炎の報告は 6 例であり，2 例が腫瘤性病変，4 例がびらん，潰瘍病変であった[1)7)～10)]．CMV の消化管病変は多彩であるが，潰瘍を形成することが多く，腫瘤を形成することは少ない[8]．潰瘍を

Fig. 3 Pathological finding of the biopsy specimen.
(a) The section shows severely inflamed duodenal mucosa with erosion and fibrosis. Atypical cells with enlarged and eosinophilic nuclei are seen in the epithelium as well as in the stroma. (H.E staining)
(b) Immunohistochemical studies reveal the infiltrating cells are strongly positive for CMV



形成する場合は，いわゆる打ち抜き様の潰瘍で潰瘍周辺には浮腫を伴わないことがほとんどであり[11]，潰瘍性大腸炎やクローン病と鑑別しにくいこともある．CMV は血管内皮細胞に感染することが多く[12]，腸管上皮細胞や腺細胞に感染することは少ないことより，潰瘍形成は血液循環障害に起因すると考えられている[13]．一方，腫瘤性病変は，消化管造影検査や内視鏡，腹部 CT にて腫瘍性病変と似た所見を呈する[8]．組織学的所見に関しては報告が少ないが，検討例では炎症性偽腫瘍様の所見を呈している[14]．すなわち，粘膜および粘膜下の浮腫や線維化，肉芽種形成，炎症細胞浸潤を認め，CMV は血管内皮細胞よりむしろ，間質の線維芽細胞や上皮細胞内への感染を特徴とする．本症例においても，血管内皮にも少数の感染細胞を認めたが，主に間質に封入体のある細胞を認め，炎症および線維化を伴っていた．

腸管の CMV 感染では，封入体は典型的な owl eye にはなりにくいといわれている[9)15)]．HIV 感染合併では，HIV 感染非合併例に比べ病変に壊死を伴う症例の割合が多く報告されている[8]．さらに HIV 感染合併例では腸粘膜の構造異型を伴うこともあり HE 染色所見のみで CMV 感染を診断するのは困難と考えられる．血中 CMV 抗原は活動性 CMV 感染症の指標となるが，CMV 腸炎での感度は 50%，CMV 網膜炎での感度は 30% と報告されており，血中 CMV 抗原の有無と CMV 感染臓器病変の有無には相関はない[8)16)]．以上のことから CMV 感染による臓器病変の最終診断には組織の免疫染色が重要である．

CMV による消化管病変は，健常人に発症した場合，抗ウイルス薬の投与なく軽快する例も報告されている．一方，高齢者や重度の消化器病変を有する症例，HIV 感染合併例では，抗 CMV 薬での加療が必要になる[17]．さらに腸管穿孔や出血，腫瘤性病変による閉塞がある場合には外科的処置が必要になる．HIV 感染に伴う CMV 腸炎では病変が多発していることや CMV による腸管の血流不全のために術後合併症も多く，緊急手術例の死亡率は 54%～87% と報告されている[18]．実際，消化管 CMV 腫瘤性病変を併発した HIV 感染症 15 例の検討では，8 例に外科的処置が施行されている．抗ウイルス薬を併用していない 6 症例のうちその後の経過が追えた 4 例はいずれも周術期の合併症にて死亡している．抗ウイルス薬を使用した症例では，外科的処置を併用した症例も含め検討可能症例 7 例中 5 例にて改善を認め，2 例が死亡している．一方，HIV 感染を合併していない消化管 CMV 腫瘤性病変 9 例の検討では，自然軽快 1 例，外科的処置が必要だった症例 4 例，抗ウイルス薬使用例（外科的処置併用例も含む）7 例であったが，いずれも再燃なく予後良好であった[8]．本症例は，腫瘤性病変が小さかったこと，十二指腸乳頭部に病巣がありながらも胆道系の閉塞がなかったことより外科的処置は行わず，valganciclovir の内服にて改善した．抗 CMV 薬の治療効果判定に関しては，血中 CMV 抗原の検出が有用であると報告されており[19)20)]，本例においても valganciclovir 開始に伴い血中 CMV 抗原は陰性化した．しかし，前述のように血中 CMV 抗原の感度，特異度には限界があり，また IRIS においては必ずしも血中 CMV 抗原は陽性化しないため，本例では valganciclovir による治療効果の判定，臨床経過の評価は，十二指腸病変では内視鏡所見，網膜病変に関しては眼底所見を重視し，血中 CMV 抗原は参考所見とした．

CMV 感染は，特に網膜症において IRIS 発症のリスクが高く，2 割前後の症例に認められるとの報告がある[21)22)]．消化管においても IRIS により腸管の穿孔を

きたした症例が報告されている[23]．十二指腸乳頭部病変に関するIRISの検討は，文献的に報告例がなかったが，本症例は炎症を伴う腫瘤性病変であったため，開始後のIRIS発症の可能性が高いと判断した．抗HIV療法開始前の日和見感染症の治療期間が1カ月未満であるとIRISのリスクが高まると報告されているため[24]，4週間のvalganciclovirによる治療後に抗HIV療法を開始した．結果，網膜病変には一度IRISを認めたが，十二指腸病変はIRISを併発することなく経過した．CMV網膜症に関しては抗HIV療法導入後8週間以上の抗CMV療法を施行していれば抗CMV薬を中止しても予後に影響がないと報告されているため[25]，IRISの治療を行った後にvalganciclovir 900mgを8週間投与し中止した．現在，当院初診後3年以上経過しているが再燃はない．

本症例は十二指腸乳頭部腫瘍と肉眼的に鑑別が困難であったが組織診断によりCMV性十二指腸乳頭部炎と診断され保存的治療が奏効した1例である．HIV感染症，移植後の免疫抑制剤の使用，抗悪性腫瘍剤の使用や膠原病に対するステロイドの使用などによる免疫不全の症例に腸管腫瘤性病変を認めた場合には，CMV感染を疑い精査を進める必要がある．さらに診断には免疫染色を含めた組織診断が重要であると考えられた．

利益相反自己申告：申告すべきものなし

文　献

1) Wilcox CM, Schwartz DA：Symptomatic CMV duodenitis. An important clinical problem in AIDS. J Clin Gastroenterol 1992；14：293—7.
2) Jacobson MA, Mills J：Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). Clinical findings, diagnosis, and treatment. Ann Intern Med 1988；108：585—94.
3) Masliah E, DeTeresa RM, Mallory ME, Hansen LA：Changes in pathological findings at autopsy in AIDS cases for the last 15 years. AIDS 2000；14：69—74.
4) Reichert CM, O'Leary TJ, Levens DL, Simrell CR, Macher AM：Autopsy pathology in the acquired immune deficiency syndrome. Am J Pathol 1983；112：357—82.
5) Drew WL：Cytomegalovirus infection in patients with AIDS. J Infect Dis 1988；158：449—56.
6) Cello JP：Acquired immunodeficiency syndrome cholangiopathy：spectrum of disease. Am J Med 1989；86：539—46.
7) Mohan H, Bal A, Garg S, Dalal U：Cytomegalovirus-associated pseudotumor simulating gastric malignancy in acquired immunodeficiency syndrome：a case report with review of literature. Jpn J Infect Dis 2007；60：134—6.
8) Kelesidis T, Tozzi S, Mitty R, Worthington M, Fleisher J：Cytomegalovirus pseudotumor of the duodenum in a patient with AIDS：an unrecognized and potentially treatable clinical entity. Int J Infect Dis 2010；14：e274—82.
9) Kim Y, Cho Y, Lee J, Jin S, Shim C：CMV infection in an HIV patient with duodenal papillitis. Endoscopy 2007；39：E23.
10) Mong A, Levine MS, Furth EE, Laufer I：Cytomegalovirus duodenitis in an AIDS patient. AJR Am J Roentgenol 1999；172：939—40.
11) 徳永博俊，毛利圭二，岡三喜男，甲斐田祐子，松本英男，平井敏弘，他：サイトメガロウイルス腸炎による小腸穿孔を契機に診断されたAIDSの剖検例．川崎医学会誌　2010；3：53—60.
12) Iwasaki T：Alimentary tract lesions in cytomegalovirus infection. Acta Pathol Jpn 1987；37：549—65.
13) 山田義也，江川直人，小澤　広，門馬久美子，榊信廣，猪狩　亨，他：AIDSの消化管病変の臨床と病理．胃と腸　1999；34：845—55.
14) Teixidor HS, Honig CL, Norsoph E, Albert S, Mouradian JA, Whalen JP：Cytomegalovirus infection of the alimentary canal：radiologic findings with pathologic correlation. Radiology 1987；163：317—23.
15) Schwartz DA, Wilcox CM：Atypical cytomegalovirus inclusions in gastrointestinal biopsy specimens from patients with the acquired immunodeficiency syndrome：diagnostic role of in situ nucleic acid hybridization. Hum Pathol 1992；23：1019—26.
16) Mori T, Mori S, Kanda Y, Yakushiji K, Mineishi S, Takaue Y, *et al.*：Clinical significance of cytomegalovirus (CMV) antigenemia in the prediction and diagnosis of CMV gastrointestinal disease after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2004；33：431—4.
17) 大中貴史，米澤昭仁，今田和典：健常成人に認められたサイトメガロウイルス胃十二指腸炎の一例．感染症誌　2013；87：49—52.
18) Michalopoulos N, Triantafillopoulou K, Beretouli E, Laskou S, Papavramidis TS, Pliakos I, *et al.*：Small bowel perforation due to CMV enteritis infection in an HIV-positive patient. BMC Res Notes 2013；6：45.
19) 花房秀次，松田文子，田中葉子，上杉妙子，太田俊彦，佐藤敏美，他：AIDSに合併したサイトメガロウイルス（CMV）感染症の早期診断及び治療効果判定におけるCMV抗原検索の有用性．感染症誌　1994；68：1105—12.
20) Roberto Manfredia, Tiziana Lazzarottob, Patrizia Spezzacatenab, Paola Dal Monteb, Antonio Mastroiannia, Olga V Coronadoa, *et al.*：Quantitative cytomegalovirus (CMV) antigenae-

mia during antiviral treatment of AIDS-related CMV disease. Journal of Antimicrobial Chemotherapy 1997 ; 40 : 299—302.
21) Murdoch DM, Venter WD, Van Rie A, Feldman C : Immune reconstitution inflammatory syndrome (IRIS) : review of common infectious manifestations and treatment options. AIDS Res Ther 2007 ; 4 : 9.
22) Kempen JH, Min YI, Freeman WR, Holland GN, Friedberg DN, Dieterich DT, *et al.* : Risk of immune recovery uveitis in patients with AIDS and cytomegalovirus retinitis. Ophthalmology 2006 ; 113 : 684—94.
23) von Both U, Laffer R, Grube C, Bossart W, Gaspert A, Günthard HF : Acute cytomegalovirus colitis presenting during primary HIV infection : an unusual case of an immune reconstitution inflammatory syndrome. Clin Infect Dis 2008 ; 46 : e38—40.
24) Haddow LJ, Moosa MY, Mosam A, Moodley P, Parboosing R, Easterbrook PJ : Incidence, clinical spectrum, risk factors and impact of HIV-associated immune reconstitution inflammatory syndrome in South Africa. PLoS One 2012 ; 7 : e40623.
25) Wohl DA, Kendall MA, Owens S, Holland G, Nokta M, Spector SA, *et al.* : The safety of discontinuation of maintenance therapy for cytomegalovirus (CMV) retinitis and incidence of immune recovery uveitis following potent antiretroviral therapy. HIV Clin Trials 2005 ; 6 : 136—46.

CMV-induced Duodenal Papillitis in a Patient with HIV-1 Infection

Rumi MINAMI, Soichiro TAKAHAMA, Eriko NAKASHIMA & Masahiro YAMAMOTO
Internal Medicine, Clinical Research Institute, National Hospital Organization, Kyushu Medical Center

We present herein a case report of a 59-year-old patient with HIV-1 infection who developed a CMV-induced pseudotumor of the duodenum. The patient presented with oral pain and dysphagia. Physical examination revealed oral thrush. An EIA and a Western blot assay for antibodies to HIV were positive. His CD4-positive lymphocyte count was initially 49/μL with an HIV viral load of 2.6×10^5 copies/mL. Cytomegalovirus (CMV) reactivation was detected with the CMV antigenemia assay. He had CMV retinitis in both eyes with unilateral blurring. An endoscopic study revealed candida esophagitis, and a tumor-like lesion with an irregular ulcer at the papilla of Vater. Histological and immunohistochemical studies revealed a CMV-induced pseudotumor and severely inflamed duodenal mucosa with infiltration of CMV-positive cells. The patient was treated with oral valganciclovir and fluconazole for three weeks. As the oral thrush and retinitis showed improvement, he began antiretroviral therapy (ART), consisting of raltegravir and TDF/FTC. One month later the patient's CD4-positive cells increased to 130/μL and the level of HIV-RNA decreased to 160 copies/mL. The CMV retinitis had transiently worsened because of an ART-induced inflammatory response, immune reconstitution inflammatory syndrome (IRIS). Six months after the ART initiation, an endoscopic study revealed that the esophagitis and the lesion at the papilla had improved. Biopsy showed no CMV-positive cells in the epithelium. The patient was now in a relatively healthy condition. CMV-induced pseudotumors of the duodenum are rare, and sometimes resemble malignancy. However, because this tomor responds to medical treatment physicians treating severely immunocompromised patients should be aware of its presentation and treatment.

〔J.J.A. Inf. D. 87 : 441～445, 2013〕