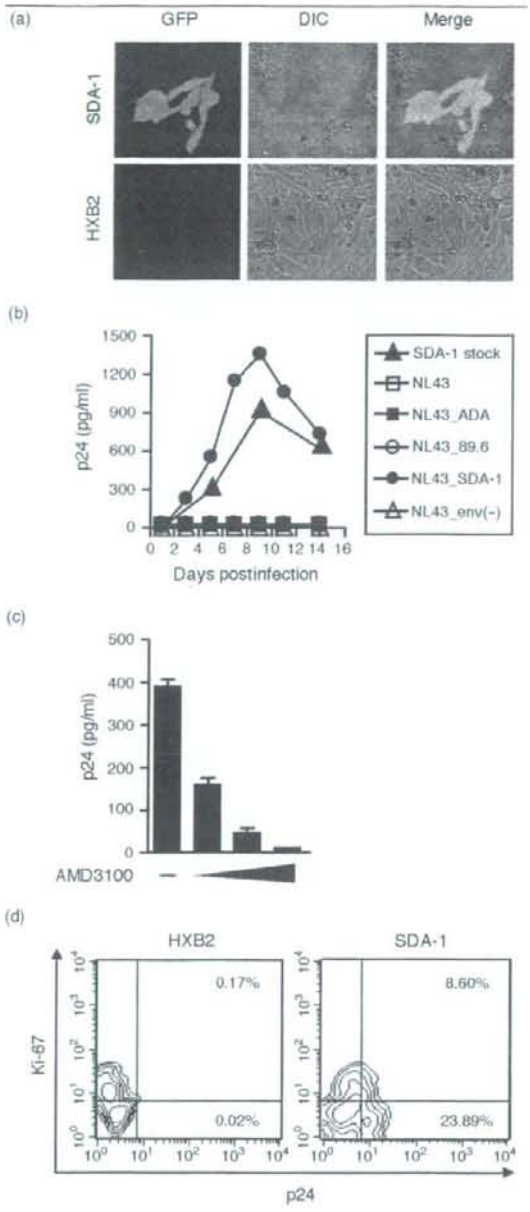

Fig. 4. SDA-1 enters and replicates in CD4$^-$ human p-hepatocytes. (a) Entry of SDA-1 into p-hepatocytes. The p-hepatocytes were exposed to the indicated HIV-1 GFP-p viruses for 48 h. Infectivity was determined as GFP$^+$ cells by confocal microscopy. (b) Replication of SDA-1 Env-chimeric viruses and SDA-1 virus stock in human p-hepatocytes. (c) SDA-1 infects p-hepatocytes through CXCR4. The inhibitory effects of AMD 3100 (0.1, 0.3 and 1.0 μM) on SDA-1 Env-chimeric viruses infection of p-hepatocytes were studied. Results shown are means of triplicate experiments. Bars, SD. (d) SDA-1 replicates in both proliferating and static human p-hepatocytes. Intracellular stainings of HIV-1-infected p-hepatocytes for p24 and Ki-67 were analyzed by flow cytometry. CXCR4, chemokine (C-X-C motif) receptor 4; GFP-p, GFP-pseudotypes.

percentage of p24 expression between Ki-67$^+$ (31%) and Ki-67$^-$ p-hepatocytes (33.1%), suggesting that SDA-1 efficiently enters and replicates in both proliferating and static hepatocytes.

Considering that SDA-1 can infect hepatocytes *in vitro*, it would have been interesting to determine whether the patient's liver was infected *in vivo*. However, consent for a liver biopsy was denied by the patient's family. There was no evidence of liver dysfunction. When virus was isolated from this patient; however, liver damage [an aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio ≥ 1] was observed at the end of the clinical stage. Although the cause of liver injury was unclear, our present data suggest that CD4-independent HIV-1 infection may lead to hepatocellular damage.

Discussion

In this study, we characterized a quasispecies of a CD4-independent HIV-1 isolate, termed SDA-1, which was able to utilize either CXCR4 or CCR5 in the absence of CD4. Moreover, we demonstrated that SDA-1 efficiently entered and replicated in Huh-7 hepatoma cells and normal human hepatocytes, through CXCR4, without inducing apoptotic cell death.

Many SIV and HIV-2 isolates can infect cells without CD4, at least to some extent. However, CD4-independent HIV-1 viruses have been rarely isolated and, so far, only a few laboratory-adapted CD4-independent HIV-1 variants have been reported. It must be noted that CD4-independent HIV-1 variants, isolated *in vitro* by passage through cells lacking CD4, have been shown to be more sensitive to neutralizing antibodies than CD4-dependent viruses [44,45]. Therefore, we might hypothesize that the emergence of a quasispecies of HIV-1 with a reduced requirement for CD4 is likely to be at a low abundance relative to the more common CD4$^+$ strains. However, with disease progression, HIV-1 variants with reduced affinity for CD4 and with increased affinity for chemokine receptor could evolve and become more robust in the viral quasispecies, disseminate in a variety of CD4$^-$ tissues *in vivo* under conditions of both reduced immunological pressure and a dramatically reduced pool of target CD4$^+$ cells concomitant with high levels of virus replication. It will be important to search the viral quasispecies in other patients, especially in the later stages of HIV-1 disease for the existence of similar CD4-independent HIV-1 variants and expanded cellular tropism.

Although the extent to which $CD4^-$ cells are infected *in vivo* is unclear, it has been widely thought to be low. Nonetheless, recent studies [11,12] utilizing the novel approach of laser capture microscopy have revealed HIV-1 sequences in isolated $CD4^-$ cells of kidney epithelium and neuronal cells, indicating that latent infection might occur in such cells or tissues *in vivo*. The mechanism of viral entry into $CD4^-$ cells remains unclear, but as we show here the evidence of emergence of CD4-independent strains *in vivo* must be kept in mind.

End-stage liver disease is now becoming a frequent cause of death in HIV-1-infected hospitalized patients. HCV and HBV coinfection with HIV-1 has been shown to enhance the progression of liver damage [16]. However, little attention has been given to the direct virological interaction between HIV and HCV/HBV in the liver, as HIV has been thought not to infect hepatocytes directly. Nonetheless, a number of reports have documented that histological liver abnormalities occurred solely as a result of HIV-1 infection. In our study, we clearly demonstrated that SDA-1 efficiently enters and replicates in both proliferating and static hepatocytes through CXCR4. To our knowledge, this is the first report that HIV-1 can efficiently replicate in normal hepatocytes. Furthermore, we have shown that HIV-1 infection did not induce significant cytocidal effects in the hepatocytes. It is noteworthy that the liver is a continuously regenerating organ. Therefore, if HIV-1 enters and integrates its DNA into the host genome, liver cells containing HIV-1 DNA will be continuously generated by the division of the infected cells. Thus, the expression of HIV-1 proteins on the infected cell surface might result in chronic damage of the liver cells by inducing host immune responses. Direct virological interaction between HIV, HCV and HBV in the liver or enhanced production of HIV-1 by inflammatory cytokines produced by the HCV and HBV-activated immune cells might also exacerbate the liver injury. At present, however, we have no definite information concerning the extent to which patients' hepatocytes harbor HIV-1 and CD4-independent HIV-1 variants.

Finally, a particularly important area of vaccine research is to take advantage of gp120 structural information to guide the design of novel envelope immunogens. As has been reported, CD4-dependent viruses hide neutralizing epitopes and only CD4 binding to gp120 induces conformational changes in gp120 to fully expose epitopes for broadly neutralizing antibodies. The CD4-independent strain we isolated here seems particularly important, as it can efficiently replicate in $CD4^-$ hepatocytes. Therefore, the gp120 structural alterations, which might expose the coreceptor binding site without binding to CD4, may also open up other sites that could yield neutralizing antibodies. Nevertheless, evidence of a clinical CD4-independent R5X4 HIV-1 virus should have important implications concerning the range of mutability and tropism of HIV-1 and the pathogenesis of AIDS.

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P.X., H.L., Y.S. and T.H. designed the study. P.X., O.U., Y.S., M.Z., Y.A. and H.G. performed the experiments. P.X., O.U, Y.S., H.L. and T.H. analyzed the data. N.S. and H.H. contributed to the coreceptor expressing cell lines. P.X., H.L., Y.S., O.U., N.S., H.H. and T.H. contributed to writing the paper. T.H. contributed to grant application and financial support.

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Analysis of Near Full-Length Genomic Sequences of Drug-Resistant HIV-1 Spreading among Therapy-Naïve Individuals in Nagoya, Japan: Amino Acid Mutations Associated with Viral Replication Activity

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Abstract

We analyzed a total of 12 near full-length genomes of drug-resistant HIV-1 spreading among therapy-naïve individuals in Nagoya, Japan. Genomes comprised seven protease inhibitor (PI)-resistant viruses possessing an M46I ($n = 6$) or L90M mutation ($n = 1$) and five non–nucleoside reverse transcriptase inhibitor–resistant viruses possessing a K103N mutation. All 12 viruses conserved both an H87Q mutation in the cyclophilin A–binding site of Gag p24 (capsid) and a T23N mutation in the cysteine-rich domain of Tat protein. PI-resistant viruses commonly possessed two cleavage site mutations in the $p6^{Pol}$/protease of Pol polyprotein (F48L in $p6^{Pol}$) and the anchor/core domains of Nef protein (L57V). These amino acid mutations represent candidates for enhancing replication activity of drug-resistant viruses and supporting expansion of such viruses in therapy-naïve individuals.

TRANSMISSION OF DRUG-RESISTANT HIV-1 in therapy-naïve individuals represents a serious problem in therapy, as such variants hinder antiretroviral therapy from the start.[1,2] Drug-resistant viruses were detected in 27 of 402 therapy-naïve patients (6.7%) in Nagoya, Japan, between 1999 and 2006.[3] Importantly, phylogenetic analysis has revealed that two main independent drug-resistant strains have been spreading in this area. One is a protease inhibitor (PI)-resistant strain possessing an M46I or L90M mutation in the protease. This strain started spreading in 2000 and was found in a total of 13 therapy-naïve patients. The other is a non–nucleoside reverse transcriptase inhibitor (NNRTI)-resistant strain possessing a K103N mutation in the reverse transcriptase. This strain started spreading in 2003 and was found in a total of five therapy-naïve patients.[3] Importantly, both strains are still growing. We recently started studying why or how these drug-resistant strains can spread in therapy-naïve individuals while maintaining drug-resistant amino acid mutations that generally confer replicative disadvantages. This study analyzed near full-length genomic sequences of drug-resistant viruses to identify clues to better understanding these epidemics.

Subjects comprised a total of 12 therapy-naïve patients. Among these, seven patients were identified with PI-resistant HIV-1 possessing an M46I mutation ($n = 6$) or L90M mutation ($n = 1$). The remaining five patients displayed NNRTI-resistant HIV-1 possessing a K103N mutation by routine genotypic drug-resistance testing from 2000 to 2006.[3] Genomic sequencing of HIV-1 was performed using plasma samples obtained at the first medical examination. HIV-1 RNA was purified from a plasma sample using a QIAamp viral RNA mini kit (QIAGEN, Tokyo, Japan). A single DNA fragment containing *gag* to *nef* genes was reverse transcribed and amplified by reverse transcription (RT)-nested polymerase chain reaction (PCR) using the Superscript III one-step RT-PCR system with platinum Taq high-fidelity kit (Invitrogen, Tokyo, Japan) and LA Taq polymerase (Takara, Shiga, Japan). Sense and antisense primers for RT-PCR were INF-13 and LTR-E, respectively. Sense and antisense primers for nested PCR were INF-12 and LTR-D, respectively. INF-19 or INF-11 primers were sometimes used instead of INF-13, and INF-20 or INF-10 primers were sometimes used instead of INF-12. Nucleotide sequences of primers were as follows: INF-13, 5′-GGT GAG TAC GCC ATT TAT TTG ACT

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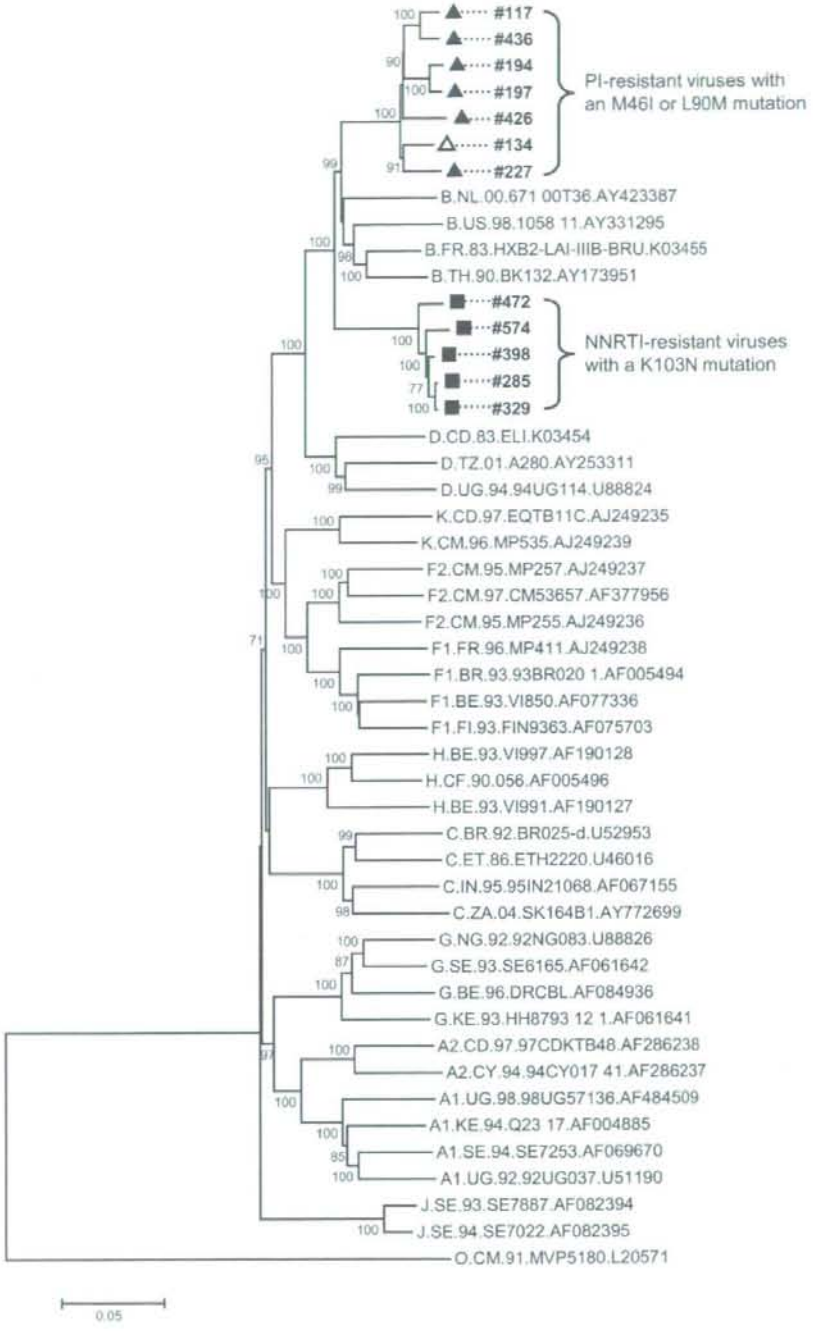

FIG. 1. Phylogenetic analysis of drug-resistant HIV-1. A phylogenetic tree was constructed using the neighbor-joining method with near full-length genomic sequences. Bootstrap values were calculated by 1,000 analyses and values greater than 70% were shown at the nodes of the tree. Scale bar represents nucleotide substitutions per site. Group O_MVP5180 was used as the outgroup. PI-resistant viruses possessing an M46I or L90M mutation are shown with closed or open triangles, respectively. NNRTI-resistant viruses possessing a K103N mutation are shown with closed squares. PI, protease inhibitor; NNRTI, non–nucleoside reverse transcriptase inhibitor.

AG-3'; LTR-E, 5'-CTT ATA TGC AGC TTC TGA GGG C-3'; INF-12, 5'-ATT TAT TTG GCG CGC GGA GGC TAG AA-3'; LTR-D, 5'-GCA TCA TTA ATT AAC CCT GGA AAG TCC CCA GCG GAA-3'; INF-19, 5'-GGT GAG TAC GCC AAA AAA CTT TTG ACT AG-3'; INF-20, 5'-AAA CTT TTG GCG CGC GGA GGC TAG AA-3'; INF-11, 5'-TCT CTC GAC GCA GGA CTC GGC TTG-3'; INF-10, 5'-GCT GAA GCG CGC ACA GCA AGA GGC GAG-3'. The RT-PCR program consisted of one cycle of RT reaction (60 min at 50°C), 1 cycle of pre-PCR (2 min at 94°C), and 40 cycles of PCR (15 s at 94°C, 30 s at 50°C, and 10 min at 68°C). The nested PCR program consisted of one cycle of pre-PCR (2 min at 94°C) and 40 cycles of PCR (15 s at 94°C, 30 s at 50°C, and 10 min at 70°C). A labeling reaction for DNA sequencing was per-

A Pol polyprotein

	p6^{Pol} ▼ Protease
AA#	48
NL4-3	VSFSF PQITL
HXB2	...N. ..V..
117	I...**L** .....
134	..LN**L** .....
194	I..N**L** .....
197	I..N**L** .....
227	..L.**L** .....
426	..LN**L** .....
436	I...**L** .....
285	....
329	....
398	....
472	....
574	....

B Nef protein

	Anchor domain ▼ Core domain
AA#	57
NL4-3	ACAWL EAQEE
HXB2	....
117	DR..**V** .....
134	DR..**V** ....D
194	D...**V** ....D
197	D...**V** ....D
227	X_1X_2..**V** .....
426	.R..**V** .....
436	DR..**V** .....
285	D.V.. ..H.D
329	D.V.. ..H.D
398	D.V.. ..H.D
472	D.V.. ....D
574	D.V.. ..H.D

C Gag p24 (capsid)

AA#	87
NL4-3	PVHAGPIAP
HXB2	.....
117	..**Q**.....
134	.A**Q**.....
194	.A**Q**.....
197	.A**Q**.....
227	..**Q**.....
426	.P**Q**.....
436	.A**Q**...X_3..
285	.A**Q**...HP.
329	.A**Q**...HP.
398	.A**Q**...HP.
472	.A**Q**...HP.
574	.A**Q**...HP.

D Tat protein

AA#	23
NL4-3	CTNCYCKKCCFHCQVC
HXB2	.....
117	.**N**.....L.....
134	.**N**.....
194	.**N**.....Y...A.
197	.**N**.....Y.....
227	.**N**.....L.....
426	.**N**.....S.....
436	.**N**.....W.....
285	.**N**.....
329	.**N**.....
398	.**N**.....
472	.**N**.....
574	.**N**.....

FIG. 2. Candidates for amino acid mutations that possibly enhance the replication activity of drug-resistant HIV-1. Protease inhibitor-resistant HIV-1 commonly possessed an F48L mutation in the carboxyl terminus of p6^{Pol} (**A**) and an L57V mutation in the carboxyl terminus of Nef anchor domain (**B**). All drug-resistant viruses displayed conservation of an H87Q mutation in the cyclophilin A–binding domain (Pro85 to Pro93) of Gag p24 (capsid) (**C**). All drug-resistant viruses also conserved a T23N mutation in the cysteine-rich domain (Cys22 to Cys37) of Tat protein (**D**). Candidate mutations are shown in bold. Cleavage points are represented as triangles in **A** and **B**. Mixed-type amino acids are represented as follows: X_1, D/A; X_2, R/C; and X_3, V/I. Amino acid sequences of NL4-3 and HXB2 were used as references. AA#, amino acid number.

formed using the BigDye terminator cycle sequencing kit (Applied Biosystems, Tokyo, Japan), and DNA sequences were determined using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). In phylogenetic analyses, multiple sequence alignment was performed using CLUSTAL W, and genetic distances were calculated based on the Kimura two-parameter model using MEGA software version 3.1.[4] Phylogenetic trees were constructed using the neighbor-joining method with 1,000 bootstrap analyses. Genomic sequences of reference HIV-1 strains were obtained from the HIV sequence database in the Los Alamos National Laboratory.[5] Recombinant formation was checked using the Recombinant Identification Program version 3.0 in the HIV sequence database.

A total of 12 near full-length genomic sequences of drug-resistant HIV-1 were successfully obtained from therapy-naïve patients. Seven PI-resistant viruses and five NNRTI-resistant viruses separately clustered together with reference subtype B viruses on a phylogenetic tree (Fig. 1). This is consistent with our previous result obtained by phylogenetic analysis using *pol* gene fragment alone.[3] We separately confirmed that they were subtype B and not recombinant forms using the Recombination Identification Program (data not shown).

We and others have previously reported that acquisition of an M46I, L90M, or K103N major mutation enables HIV-1 to survive under pharmacotherapeutic pressure but simultaneously sacrifices the replicative activity of such viruses in the absence of drug.[6,7] This fact forced us to hypothesize that our drug-resistant viruses restored reduced replication activity by acquiring some mutations in the genome, which thus consequently survive and expand under drug-free conditions such as in therapy-naïve patients. We therefore extensively searched for candidate amino acid mutations that might offer advantages in viral replication, revealing four interesting mutations.

The first was an F48L mutation located in the carboxyl terminus of $p6^{Pol}$, and the second one was an L57V mutation located in the carboxyl terminus of the Nef anchor domain (Figs. 2A, 2B). These were specified in the PI-resistant HIV-1 strain. Findings of A431V, L449F, and P453 mutations in the p7/p1 and p1/$p6^{Gag}$ cleavage sites of Gag polyprotein, and associated restorative activities on viral replication of PI-resistant HIV-1 have been reported,[8,9] but our viruses displayed no such mutations. The F48L mutation in $p6^{Pol}$ and/or the L57V mutation in the Nef protein might have restoration activity in our PI-resistant viruses.

The third was a non–cleavage site mutation found in Gag p24 (capsid). Several amino acid mutations in the non–cleavage site of Gag polyprotein have been reported to restore reduced replication activity of PI-resistant HIV-1.[10] One of these is an H219Q mutation also known as an H87Q mutation in the capsid. Amino acid 87H is located in the cyclophilin A–binding site, and the H87Q mutation reduces incorporation of cyclophilin A into HIV-1 virions, thus elevating HIV-1 replication.[11] Interestingly, all our drug-resistant viruses commonly possessed the H87Q mutation in the capsids, suggesting that replicative activities were enhanced by this mutation (Fig. 2C). Notably, in addition to the H87Q mutation, V86A/P, I91H/V, and A92P mutations were frequently found in the cyclophilin A–binding site. These additional mutations may also be associated with viral replication activity through binding modulation to cyclophilin A.

The fourth was again a non–cleavage site amino acid mutation found in the cysteine-rich domain of Tat protein. A previous study reported T23N as a polymorphic mutation that increased Tat transactivation activity on HIV-1 provirus gene expression.[12] Interestingly, all our drug-resistant viruses also commonly possessed this T23N mutation (Fig. 2D). Elevated Tat activity may plausibly support the replication of drug-resistant viruses.

As another interesting mutation, we found an insertion mutation of RPEP in the PTAPP motif of $p6^{Gag}$ in four cases of NNRTI-resistant HIV-1 (data not shown). At present, whether this insertion mutation confers any advantage for NNRTI-resistant viruses to survive under drug-free conditions is unclear.[13–15]

In conclusion, we successfully found primary candidates of amino acid mutations that might enhance the replicative activity of drug-resistant HIV-1 for surviving under drug-free conditions. Further investigations are required to elucidate whether these mutations substantially support the replication of drug-resistant viruses. Preliminary findings from our recent experiments have demonstrated positive roles of such mutations, particularly for H87Q mutation in the capsid.

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原　著

HIV 脳症 5 例の臨床的特徴と経過

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要旨：HIV 脳症 5 症例を報告した．1996 年から 2005 年 11 月の間に名古屋医療センターを受診した HIV 感染症 458 症例（うち AIDS は 127 症例）を対象とした．HIV 脳症と診断した症例はいずれも高度の免疫不全状態にあり，他の日和見感染症を 3 症例にみとめた．4 症例は HIV 感染症が判明したのとほぼ同時期に HIV 脳症と診断された．5 症例とも HIV に対して抗ウイルス療法は未施行であった．HAART を施行することで全例で症状の改善をみとめ，死亡はみとめなかった．精神科介入を要したり 1 例を除いて社会復帰できないなど，行動障害を呈した HIV 脳症の機能予後は不良であり，HAART のみの治療効果は不十分と考えられた．

（臨床神経，48：173—178，2008）

Key words：HIV，AIDS，認知障害，行動異常，予後

はじめに

HIV 感染症は病期が進行するにつれ日和見感染症など各種疾患を合併する．なかでも HIV 脳症は AIDS 指標疾患の 1 つであり，中枢神経領域における重要な合併症として挙げられる．亜急性から慢性に進行する記銘力低下，注意や意欲の低下，思考緩慢といった認知障害と，動作緩慢や失調性歩行などの運動障害を呈し，頭部 MRI T_2 強調画像や FLAIR 画像にて大脳白質から基底核にかけてびまん性の高信号を生じ皮質下は保たれることを特徴とする[1]．しかし我が国では HIV 脳症の臨床報告は非常に少ない．そこでわれわれは，HIV 東海北陸ブロック拠点病院である当院で経験した HIV 脳症の自験 5 症例について，その臨床的特徴と経過について検討した．

対　象

1996 年から 2005 年 11 月に名古屋医療センター（以下当院）内科を受診した HIV 感染症のうち，神経内科に紹介された症例のうち HIV 脳症と診断されたものを対象とした．

方　法

当院内科より神経内科を紹介受診した HIV 感染症の症例に対し，著者の神経内科医 2 名によって神経学的診察，髄液検査，頭部 MRI を施行した．認知障害と運動障害の双方をみとめ，血液検査，髄液検査，各種画像検査にて代謝異常や日和見感染症，悪性腫瘍等が除外されたものを HIV 脳症と診断し，神経学的所見，長谷川式簡易痴呆スケール（以下 HDS-R）もしくは Mini-mental State Examinaton（以下 MMSE），CD4 陽性細胞数（以下 CD4）および血清 HIV ウイルス量，頭部 MRI にて経過を追跡した．

結　果

上記期間に累計 458 症例の HIV 患者が受診し，そのうち AIDS 発症者は 127 症例であった．AIDS のうち 25 例に中枢神経合併症をみとめた．中枢神経合併症の内訳は Table 1 にまとめた．HIV 脳症は 5 例にみとめた．HIV 感染症の感染経路は 5 例とも同性間性行為と推定された．全例で HAART を施行し，CD4 の改善と HIV ウイルス量の抑制をみとめた．以下，症例を提示する．

症例 1　37 歳男性

職業はデザイン関係．2003 年 8 月から微熱と歩行障害が出現し，同年 9 月に動けなくなり前医に入院した．10 月に尿閉が出現．頭部 MRI では大脳，脳幹にびまん性病変があり，ADEM もしくはウェルニッケ脳症と診断され，ステロイドパルス療法とビタミン B 大量を投与されるも効果なし．その後，HIV 抗体陽性と判明したため，12 月に当院に転院した．体温 38.6℃，臥床状態で，四肢の関節腫脹があった．自発的に開眼し，寡動．発語は「イタイ」など限られた単語のみであった．知能は HDS-R は 1 点（場所について「病院」を選択できた），WAIS-R は判定不能．脳神経はほぼ正常であり，運動は指示にしたがえず評価不能，両側に強制把握をみとめた．上肢

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[3]同　臨床研究センター
（受付日：2007 年 5 月 11 日）

Table 1　Complications of HIV infection in central nervous system

	-July 2000	August 2000-April 2003	May 2003-November 2005
Toxoplasmic encephalitis	3	1	2
PML	2		1
Cryptoccocal meningitis	1	1	1
Primary CNS lymphoma	1	1	
HIV encephalopathy			5
Tuberculous meningitis			1
Cytomegaloviral encephalitis			1
Other		Unknown 1 Amebic encephalitis 1	Viral meningitis 1 Hydrocephalus 1
Total	7	5	13
HIV infectious cases (AIDS cases)	106 (19)	231 (56)	363 (127)
Rate of CNS complications with AIDS	36.8%	8.9%	9.4%

PML: Progressive multifocal leukoencephalopathy

Table 2　Counts of CD4+ lymphocytes and HIV viral load before and after HAART

	Case 1	2	3	4	5
CD4+ lymphocytes (before → after HAART) (/μl)	71 → 276	11 → 281	8 → 74	17 → 244	5 → 259
HIV viral load (before → after HAART) (copies/ml)	2.1×10^5 →< 50	5.3×10^5 →< 50	5.3×10^5 →< 50	1.2×10^7 → 1.2×10^3	1.3×10^6 →< 50

Table 3　Findings of cerebrospinal fluid at time of HIV encephalopathy diagnosis

	Case 1	2	3	4	5
Cell counts (/μl)	2	5	7	3	47
Protein (mg/dl)	45	46	50	25	21
Glucose (blood glucose) (mg/dl)	29 (80)	48 (100)	48 (126)	48 (96)	85 (244)
β-2 microglobulin (μg/ml)	7.1	—	—	3.5	3.7
HIV viral load (copies/ml)	5.9×10^4	—	1.7×10^4	1.8×10^3	9.3×10

—: not examined

に振戦があり，四肢に筋強剛をみとめた．腱反射は全体に減弱し，バビンスキー徴候は両側陽性．尿閉のため尿道カテーテルが留置されており便失禁状態であった．血液検査（Table 2）ではCD4 71/μl，HIVウイルス量 2.1×10^5copies/ml，HBs抗原陽性であり，HCV抗体，梅毒，β-D-グルカン，サイトメガロウイルスC10/C11抗原，トキソプラズマIgM/IgG抗体，クリプトコッカス抗原はいずれも陰性であった．髄液検査（Table 3）では細胞数2/μl，蛋白45mg/dl，糖29mg/dl，HIVウイルス量 5.9×10^4copies/ml．β-2ミクログロブリン7.1μg/ml．墨汁染色陰性，結核菌，非定型抗酸菌，サイトメガロウイルス，JCウイルスのPCRはすべて陰性であった．また，一般細菌，抗酸菌，真菌培養はいずれも陰性で，細胞診も陰性であった．脳波は6～7Hzの全般性徐波をみとめた．神経伝導速度では上肢は筋電図混入が強く判定不能で，下肢はF波をふくめ正常であった．入院時頭部MRI（Fig. 1）では，脳幹および大脳白質にびまん性に広がる高信号域をみとめた．

以上から，HIV脳症と診断し，発熱の原因はカテーテル留置にともなう尿路感染症と思われた．2004年1月よりHAARTを施行し，その約1カ月後より発動性と運動障害は改善したが，下肢関節は拘縮変形のため立位歩行はできなかった．6カ月後のHDS-R 17点，WAIS-Rは言語性IQ 88，動作性IQ 69，全体IQ 77．20カ月後にはHDS-R 22点と，認知機能障害は不完全ながらも徐々に改善傾向を示した．HAART開始1カ月後においてCD4 201/μl，HIVウイルス量 2.3×10^3copies/mlと改善し，22カ月後ではCD4 226/μl，ウイルス量は検出感度以下とさらに改善した．しかし，人格変化がいちじるしく，周囲に対して攻撃的言動をとったり，夜間大声で叫ぶなどの精神症状が強かったために精神科介入による投薬をおこない，約1年後に施設入所となった．頭部MRIの経時変化をFLAIR画像（Fig. 1）にて検討すると，両側左右対称性の大脳前頭葉から基底核にかけて白質の萎縮が進行していた．

症例2　35歳男性

27歳時に梅毒の既往がある．2004年6月，乾性咳嗽，労作

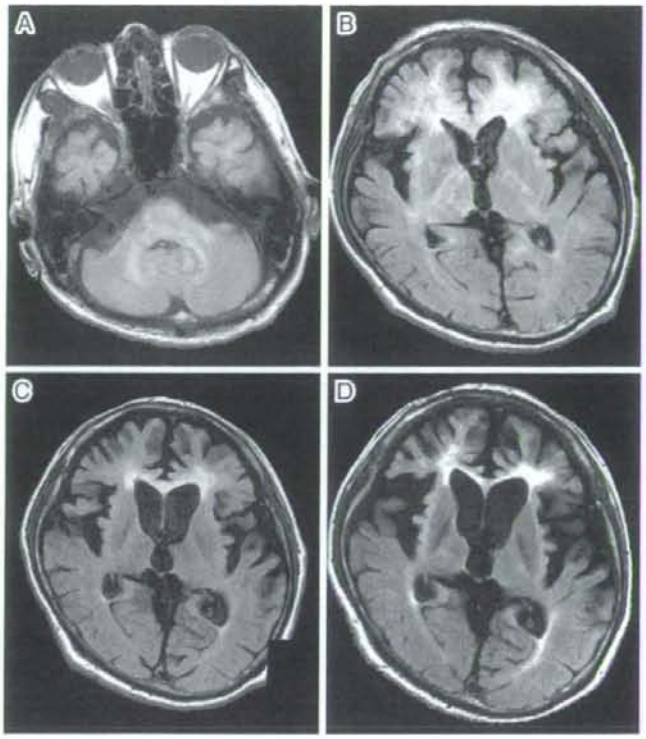

Fig. 1 Brain MRI (FLAIR) of case 1
A and B which were done at admission, revealed a diffuse lesion with high signal in the brain stem, cerebellar peduncles and cerebral white matter, atrophy of the basal ganglia and frontal lobes. C and D, which were done at 6 and 31 months respectively after HAART, showed that diffuse cerebral atrophy in the white matter had remarkably progressed.

時呼吸苦が出現．同年 8 月，発熱，呼吸苦にて前医入院した際に HIV 抗体陽性と判明し，カリニ肺炎と診断され治療を受けた．その頃からものわすれ，ふらつきを自覚．同年 9 月，当院に転院した．転院時，発動性の低下，動作緩慢，起居動作や歩行時にふらつきがあり，つぎ足歩行は不可能であり，その他の脳神経や筋力，感覚，腱反射には異常をみとめなかった．神経心理検査では，HDS-R 24 点，WAIS-R は言語性 IQ 86，動作性 IQ 65，全体 IQ 74 と認知機能障害をみとめた．また，50 音表を書くのに 80 秒かかり，立方体模写はできないなど，動作速度の低下や空間能力低下をみとめた．カリニ肺炎治療終了の 1 カ月後より HAART を開始したが，ふらつきの悪化，部屋をまちがえたり，前日のでき事を忘れたりといったことがしだいにめだつようになった．入院 1 カ月後に施行した頭部 MRI にて T_2 強調画像で両側前頭葉白質に左右対称性にびまん性に広がる高信号域をみとめ，HIV 脳症と診断した．HAART 開始 5 カ月後の神経学的診察では異常をみとめなかったが，MMSE は 28 点と認知機能障害の残存をみとめた．診断から約 1 年後，不安焦燥感を執拗に訴えて本人の強い希望で緊急入院したが，入院翌日に院内備品を持ち出したところを地下鉄職員に発見され，窃盗のうたがいで警察の事情聴取を受けた．その後，外来を不定期受診している．

症例 3　51 歳男性

職業は会社経営．2004 年 5 月に全身倦怠感と発熱が出現．同年 6 月に異常言動と失見当識が加わりしだいに傾眠となったため前医に入院し，HIV 抗体陽性と判明したため 7 月，当院に転院した．転院時 MRI では T_2 強調画像にて両側基底核に小病変をみとめた．当初，意識障害の原因としてクリプトコッカス髄膜炎，トキソプラズマ脳炎をうたがい治療を開始したが，血清および髄液中のクリプトコッカス抗原ならびに血清トキプラズマ抗体は陰性であった．血液培養から非定型抗酸菌が検出され，発熱は非定型抗酸菌敗血症によるものと診断し抗菌剤にて改善した．また，CD4 4μl と低値であったため HAART を施行した．その後，意識障害は改善し，尿失禁を残すも他の日常生活動作は自立となり約 4 カ月後に退院した．退院前後よりしだいに躁状態となり，入院中に高価な身の回りの品を換金して無断外出をしたり，退院後は妻が外出している際に知人と旅行に行くといった行動障害が出現した．服薬アドヒアランスも不良となり，ふらつきが悪化し歩行困

難となるなどの運動機能障害が悪化したため再入院となった．再入院時，動作緩慢，失調性歩行で両側バビンスキー徴候陽性，尿失禁をみとめ，HDS-R 19点であった．血液検査はCD4 171/μl，HIVウイルス量は感度以下であった．髄液検査および頭部MRIでは日和見感染症を示唆する所見はみとめず，経過からHIV脳症を当初より合併していたと考えHAARTを継続した．深夜に家族と偽って知人を病室に招き入れるなど病棟のルールを守れずに強制退院となった．その後は精神科外来にて抗躁薬と抗精神病薬を投与し，徐々に落ち着きを取りもどしたが，HDS-Rは20点前後で推移している．

症例4　28歳男性

職業は代用教員．18歳時にバーキットリンパ腫に対し自己末梢血幹細胞移植を受け治癒している．2005年5月ころからものわすれを自覚した．同年6月，バーキットリンパ腫の経過観察のため施行した血液検査で汎血球減少を指摘され，前医に入院しHIV抗体を測定したところ，陽性と判明した．同年7月，職場で倒れているのを発見され救急車にて当院を受診した．体温37.7℃，朦朧状態で物品呼称および理解は比較的保たれているが復唱はできず，上肢の観念運動失行，右同名半盲，右注視麻痺，構音障害，右不全片麻痺，バビンスキー徴候右陽性をみとめた．入院時頭部MRIではT_2強調画像にて左右対称性びまん性の白質病変をみとめた(Fig. 2)．入院3日目より右片麻痺，失語は急速に改善し，入院1週間後の診察では失見当識をみとめるが失語や麻痺は消失していた．動作緩慢であり，50音表の書き取りに105秒かかった．MMSEは22点，立方体は模写できなかった．WAIS-Rは言語性IQ 84，動作性IQ 79，全体IQ 80と低下しており，空間能力低下，短期記憶障害などの認知機能障害をみとめた．SPECTでは両側前頭葉の血流低下に加え，左頭頂葉付近の血流増加をみとめ，脳波では左前頭部に棘波をみとめた．運動機能障害と認知機能障害をみとめ，頭部MRIでも白質病変をみとめることから，亜急性にHIV脳症を生じており，今回の入院契機であった一過性の大脳左半球症状はてんかん様発作であった可能性が考えられた．その後外来にてHAARTを施行した．発症より約6カ月後，見当識は良好だが時に単語がすっと出てこないことがある．運動障害はなく，HDS-Rは27点，MMSEは25点，50音表の書き取りは35秒で可能だがラ行が抜けていた．立方体模写は可能となった．HAART前後での頭部MRIを比較すると，わずかに病変は縮小しており，画像検査上もHIV脳症の改善をみとめた．転職し社会復帰を果たしている．

症例5　63歳男性

職業は会社員．2005年8月に微熱と全身倦怠感，体重減少を自覚した．10月初旬より上記に加えて湿性咳嗽，見当識障害，夜間せん妄が出現した．10月中旬に体重減少と呼吸苦の精査にて前医入院し，胸部CTにて間質性肺炎，胃内視鏡下生検にてサイトメガロウイルス胃炎と判明し，HIV抗体陽性であったため当院に転院した．転院時，呼吸不全をみとめ，神経学的診察では，軽度意識障害（Japan Coma Scale-2），自発性

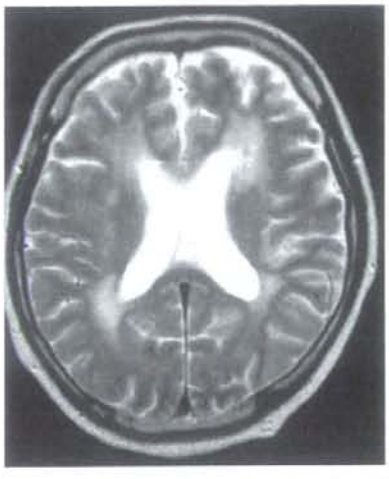

Fig. 2　Brain MRI (T2 weighted image) of case 4
It revealed that the diffuse high intensity area in cerebral white matter spared the subcortex.

低下，動作緩慢をみとめ，指鼻試験および膝踵試験は拙劣で，起居動作や歩行時にはふらつきがあり，つぎ足歩行はできなかった．立方体模写は不可能で，1から26の数唱に26秒，同じく書き取りに36秒かかった．50音表の書き取りは途中で止まってしまい遂行できなかった．全身状態が安定した後に施行したHDS-Rは12点，WAIS-Rは言語性IQ 62，動作性IQ 52，全体IQ 55と著明な低下をみとめた．頭部MRIにて，橋と両側大脳前頭葉白質から脳梁にT_2強調画像でびまん性の高信号域をみとめた．当院転院後，ST合剤およびステロイドによるカリニ肺炎の治療を施行し呼吸状態は改善したが，神経学的には変化がなかった．11月よりHAARTを施行したところ直後に一過性の譫妄をきたしたが，開始1週間後より病室で小説を読み，徐々に他の症状も改善していった．HAART開始1カ月後の診察では意識清明，歩行は自立しているがつぎ足歩行は不可能であった．HDS-R 29点，MMSE 30点と改善をみとめた．50音表書き取りは「な」行でとまってしまった．2カ月後に自宅退院され，現在も外来にてHAARTを施行している．

考　察

HIV感染症は近年，HAARTをはじめとする治療法の進歩によって当初恐れられていた日和見感染症や悪性腫瘍は減少傾向を辿っており[1,2]，中枢神経合併症も同様の傾向を呈している[4)~7)]．しかし，HIV脳症はHAARTによっても発症頻度が減少しないとされ[8,9]，その理由としてはHAARTによってHIV脳症をふくめたAIDS症例全体の生命予後が改善することが指摘されている[9]．

今回のHIV脳症5例について，診断時のCD4とHIVウイルス量をTable 2にまとめた．いずれもCD4は200/μl以下

で平均 22.4/μl ときわめて低値であり，諸外国での HAART 導入以前と同様の傾向を示している[8]．他の日和見感染症を合併しており，高度の免疫不全状態であったと思われた．

次に，HIV 脳症診断時の髄液検査所見については，Table 3 に示すように症例 5 を除いていずれも細胞数は正常（症例 5 についても 1 週間後の再検査時には正常），蛋白は正常から微増であった．測定しえた症例では，髄液中 β-2 ミクログロブリンはいずれも 2μg/ml を超えていた．髄液中の HIV ウイルス量はばらつきが多いものの症例 1，3，4 では血液中のウイルス量と比較しても高値であった．髄液中の糖は全症例とも低値を示した．HIV 脳症において髄液中の細胞数増多や蛋白の上昇がときにみとめられることは知られているが，髄液中の糖についてはあまり検討がなされておらず，Navia らが HIV 脳症 41 症例中 1 例のみ糖が低値であったと報告している[1]．われわれが経験した 5 症例において，頭蓋内の細菌感染症は髄液培養検査が陰性であったことや経過から否定的であり，髄液中の糖が低値であった理由は不明であった．

HIV 脳症の症状は運動，認知，行動の 3 つに大別される[1]．今回の 5 症例において，運動障害と認知障害は程度の差異はあるものの全症例にみとめられたが，行動異常の有無については症例差がいちじるしかった．運動機能障害については動作緩慢は全症例とも改善をみとめた．失調は完全消失にはいたらないものの改善傾向であり，結果として関節炎による関節拘縮をきたした症例 1 以外を除いては日常生活動作が自立となっており，運動機能予後は良好と思われた．

認知機能について，経過中に適宜施行した HDS-R もしくは MMSE の結果からはいずれの症例も追跡しえた範囲では改善傾向にあり，症例 1 は 31 カ月を経過した時点でもなお改善傾向にあるが，依然障害は残存している．

認知障害とならんで，行動障害は服薬アドヒアランスを大きく低下させ療養を困難とする要因となった．症例 1，2，4 では経過中に顕著な行動異常が出現し，今回の症例で唯一症例 4 のみが就労を果たした．他の症例と比較すると認知機能障害の残存はみとめていたものの，診断当初より無気力をはじめとする行動障害をともなっていなかったことがその要因と思われた．このことからは，HIV 脳症に特徴とされる運動機能障害，認知機能障害，行動障害のうち，行動障害が強いばあいには就労は困難となりうることが示唆された．

HIV 脳症の治療として，全症例ともできるだけ早期に HAART を導入した[10][11]．今回追跡しえた期間内は死亡をみとめず，他の中枢神経疾患が多くのばあいに致死性である[7][12]ことを考えると，HIV 脳症の短期間の生命予後は良好であると思われた．その一方で機能予後は不良と考えられ，HAART のみでは治療効果は不十分であると思われた．今後，HAART に加えてあらたな治療の確立が望まれる[13][14][15]．

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Abstract

Clinical features and courses of 5 cases with HIV encephalopathy

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Clinical features and courses of 5 cases with HIV encephalopathy were reported. The subjects were among the patients registered as HIV patients at the Nagoya Medical Center, between 1996 and 2005. There were 458 patients with HIV infection including 127 cases of AIDS. All patients suffered from severe immunological deficiency when HIV encephalopathy developed. Other opportunistic infections had also occurred in three patients. HIV encephalopathy was one of the presenting manifestations of HIV infection in four patients, and no patients had received antiretroviral therapy. HAART improved motor disturbance and their ADL became independent except for one case. Improvements in neuropsychological examination scores were noted in all cases. Recovery from psychiatric symptoms, however, was incomplete. Four patients could not work, and 3 needed psychological treatment due to behavioral abnormalities. HIV encephalopathy is not a lethal disease but the functional prognosis was very poor. New therapy is needed for HIV encephalopathy.

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Key words: HIV, AIDS, cognitive impairment, behavioral change, prognosis

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