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Author(s)	Jiranan, Warachit
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氏　　名	Jiranan (ジラナン) Warachit (ワラチット)
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学 位 論 文 名	Aberrant life cycle of human immunodeficiency virus type 1 CRF15_01B-like clinical isolates from Thailand in human CD4$^+$ T-cell lines （ヒト CD4＋T 細胞株細胞において異常な生活環を示すヒト免疫不全ウィルス１型（HIV-1）のタイ CRF15_01B 臨床分離株）
論 文 審 査 委 員	（主査） 教　授　生田　和良 （副査） 教　授　松浦　善治　　教　授　塩田　達雄

論 文 内 容 の 要 旨

〔　目　　的　〕Objective

Human immunodeficiency virus type 1 (HIV-1) has been classified into major (M), outlier (O), and non-M/non-O (N) groups. Within group M there are at least nine genetically distinct subtypes. Also, increasing numbers of circulating recombinant forms (CRFs) have been identified by phylogenetic analysis. The vast amount of information on HIV-1 has been accumulated by studies using subtype B. However, the majority of HIV-1 infections around the world are caused by non-B subtypes. Evidence is accumulating of the effects of HIV-1 subtype diversity at least in the sensitivity to antiretroviral drugs, drug resistance, and viral fitness. Therefore, it is imperative to study more about non-B subtypes as well and better understand their virology. In this study, we examined the virological characteristics of 4 Thai HIV-1 isolates which were classified as CRF01_AE, based on the reactivity with synthetic subtype E- and B-specific Env V3 peptides, in comparison with 2 subtype B Japanese isolates.

〔　方法ならびに成績　〕Methods and Results

A total of 6 HIV-1 clinical isolates were used：0-47-1, CU98-26, CU98-28, and CU98-31 from Thai and 0-4-26 and 17-3-6 from Japanese. The CU98-26, CU98-28, and 0-4-26 were derived from an asymptomatic phase. The others were derived from an AIDS phase. These viruses were isolated by co-culturing with phytohemagglutinin (PHA)-stimulated PBMC and use as inocula for the experiment. The human CD4$^+$ T-cell lines MT-4 and MOLT-4 were infected with these HIV-1 clinical isolates at the same amounts. As controls, the cell lines were mock-infected or similarly infected with the same amount of the subtype B laboratory strain LAI. During 30 days after infection with these isolates, severe or slight cytopathic effects were detected. The viral production rates in the CRF01_AE-infected cells were variable, although the 2 subtype B isolates showed similar

production rates in both T-cell lines, as in LAI. While infections with the 3 CRF01_AE isolates (0-47-1, CU98-26, and CU98-31) in MT-4 and the 2 CRF01_AE isolates (CU98-26 and CU98-31) in MOLT-4 showed the apparent production of viral particles at an acute phase of infections, both cell lines infected with CU98-28 and MOLT-4 cells infected with 0-47-1 produced undetectable levels of HIV-1 particles. All these infected cells were maintained by regular passage every 3 days for more than 1 year. The cell proliferation rates of all the infected cells were stable. Overall, the data indicate that the characteristics of the HIV-1 life cycle clearly differ between subtype B Japanese isolates and the Thai isolates, except for CU98-26 in both cell 1ines and 0-47-1 in MT-4 cells, whose characteristics were very similar to those of the subtype B.

Next, we examined the presence of HIV-1 DNA in infected cell lines during the persistent phase. First, the integrated form of viral DNA was examined by Alu-PCR. In addition to the chronically infected cell lines, MOLT-4 infected with 0-47-1, which was completely virus non-producing, also showed amplified bands with a similar intensity, indicating a normal level of integrated viral DNA with no viral expression. However, in the other non-producer cells (CU98-28 and CU98-31 in both cell lines), the HIV genome was detectable using different primer at LTR and *gag* regions but unable to detect the integration form of HIV-DNA. Then we performed the Inverse-PCR of their low-molecular weight (LMW)-DNA using primers located at the U3 of LTR (Rev-Nef-4) and *gag* (LA1) showed a band of 2-LTR from all chronically and aberrantly infected cells, except the 0-47-1-infected MOLT-4. Thus, the aberrantly CRF01_AE-infected cells (CU98-28 and CU98-31) carry the extrachromosomal circular (ECC) form, not the integrated form. Comparison of the Env V3 region between individual isolates derived from infected MT-4 and MOLT-4 cells with the viruses derived from PBMCs, which were used as the initial viral stock, revealed CU98-26, CU98-28, and CU98-31 to be greatly different from the original stock. Surprisingly, the sequences of the above 3 isolates of CRF01_AE in T-cell lines were very similar to those of subtype B. However, 0-47-1 in T-cell lines as well as in PBMCs showed similarity to other 3 CRF01_AE isolates in PBMCs. In contrast, when we used the full-length Vpu sequence to make a phylogenetic tree, all these 3 CRF01_AE isolates including 0-47-1 in PBMCs as well as in T-cell lines were clearly separated from the subtype B isolates in PBMCs and T-cell lines. Therefore, we next determined the near full-length sequence of CU98-26 in MT-4 and MOLT-4 cells and compared it with the sequences of subtype B, subtype C, and CRF01_AE as controls using the Simplot package. CU98-26 in both cell lines displayed distinct mosaic patterns, with multiple breakpoints between CRF01_AE and subtype B which were very similar between infected MT-4 and MOLT-4 cells：subtype B sequences at Pol Integrase to Vif as well as most parts of Env gp120 and Nef to LTR.

〔 総　　括 〕General Summary

Infections of 4 CRF01_AE clinical isolates (AE; 0-47-1, CU98-26, CU98-28, and CU98-31) were examined in human $CD4^+$ T-cell lines, MT-4 and MOLT-4. The CU98-26 in both cell lines and 0-47-1 in MT-4 established chronic productive infections, as in 2 control isolates of subtype B, while 0-47-1 in MOLT-4 caused a latent infection. In contrast, CU98-28 and CU98-31 established aberrant infections in both cell lines with undetectable level of integration form but carry ECC form of HIV-DNA. Interestingly, analyses based on phylogenetic trees and sequencing revealed that all the AE isolates, except 0-47-1, displayed CRF15_01B-like mosaic structures of AE with B sequences in several regions that were apparently different from those of the inocula originally used. Thus, in infections of most of the CRF01_AE isolates, it is understood that a minor population with mosaic patterns having multiple breakpoints between CRF01_AE and subtype B-like regions in the inocula were picked up by the T-cell lines.

論文審査の結果の要旨

HIV-1は、幾つかのサブタイプ、さらにサブタイプ間による組換え型に分けられる。今回の研究は、日本人由来の２株とタイ人由来の４株の臨床分離株について、ヒトCD4$^+$ T細胞株（MT-4とMOLT-4）への感染様式を比較検討したものである。ウイルス分離を行った感染者の血清抗体からは前者はB型、後者はCRF01_AE型と判定されたものである。結果は、前者２株と後者の１株が、これまで知られていたB型と同様、ウイルスを産生する慢性感染を引き起こした。また、後者の１株はMT-4で慢性感染を、MOLT-4で潜伏感染を引き起こした。残る後者２株は、インテグレーションせずに環状型DNAとして長期間存続していた。遺伝子配列を解析した結果、近年タイにおいて増加傾向にあるAE型とB型間のモザイク型の構造を取っているものが、今回のタイ分離株の内３株にも認められた。以上、タイHIV-1の特徴を明らかにしたもので学位論文に値する。平成19年２月２日、最終試験を口頭で行った結果、合格と判定した。