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学位授与の要件	学位規則第４条第２項該当
学位論文名	Immunofluorescence assay in India for confirmation of HIV-1 infection using a T-cell line infected with defective HIV-1 （不完全HIV-1に感染したT細胞株を用いた蛍光抗体法によるインドでのHIV-1感染確定試験について）
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論文内容の要旨

〔　目　　的　〕

In India, the enzyme immunoassay (EIA)/rapid test is used for screening and confirmatory antibody testing of HIV infection, following a serial testing strategy, as recommended by the National AIDS Control Organization (NACO), India and the World Health Organization. All HIV reactive samples are further confirmed by two other rapid tests working on different principles. However, the US Centers for Disease Control and Prevention recommend that all reactive screening tests be confirmed by supplemental testing using either a Western blot (WB) or indirect immunofluorescence (IF) assay. The goal of the present study was to explore the suitability of human T-cell line persistently infected with defective HIV-1 (L-2 cell clone) as a potentially useful source of viral antigens in IF assays for HIV confirmatory testing. The advantage of using this cell clone is that L-2 cells show exceptionally strong HIV-1 antigen expression compared with other persistently HIV-1 infected human T-cell lines and continuously produce non-infectious, reverse transcriptase negative doughnut-shaped particles.

〔　方法ならびに成績　〕

Methods: A total of 2104 sera were obtained from specimens received from individuals attending Integrated Counseling and Testing Centre (ICTC) of a state reference laboratory in India and samples collected during a sentinel surveillance program. These sera were tested for the presence of HIV-1 antibody using EIA/rapid tests, according to the guidelines of the NACO, India, and were also subjected to IF test using L-2 cells. MT-4 cells were used as control cells. The L-2 and MT-4 cells were maintained in Department of Virology, Research Institute of Microbial Diseases, Osaka University, Japan. The typical cytoplasmic staining patterns of L-2 cells were graded from 0 to +4 according to the intensity of fluorescein isothiocyanate in approximately 25% of L-2 cells present in a monolayer of the HIV-infected wells. WB and a nested reverse transcriptase polymerase chain reaction (RT-PCR) with primers targeting the C2–V5 fragment of the env gene, were performed on discrepant samples. An infectious molecular clone of Indian subtype C HIV-1, Indie-C1 was used as a positive control for RT-PCR.

Results: IF assay results were 100% concordant with EIA/rapid tests for 212 HIV-1 positive samples and 1889 HIV-1-negative samples. Interestingly, three (0.14%) samples negative by EIA/rapid tests were weakly or moderately positive (1+/2+) by IF test. The positive reactions of the three sera by IF test were confirmed on repeat testing. All three of these samples were confirmed to be negative by WB (reactive with Gag/Pol, but not with Env). When these 3 plasma samples were subjected to RT-PCR analysis for detection of HIV-1 RNA in plasma, it was found that all the samples were positive by this analysis These three samples were from individuals who voluntarily reported for HIV testing because of high-risk practices, and they may have been at an early stage of HIV infection.

〔　総　　括　〕

These results confirm that the IF test using L-2 cells being slightly more sensitive than WB, is a cost-effective, sensitive and specific alternative method for confirmation of HIV-1 infection and could be included in the diagnostic algorithm in reference laboratories in developing countries.

論文審査の結果の要旨

HIV感染の有無を調べる検査方法として、インドではNational AIDS Control Organization (NACO)が推薦するEIA迅速診断法が採用されている。確定診断においても、別の原理に基づく迅速診断法が採用されている。しかし、アメリカCDCは、確定診断としてウェスタンブロット法もしくは蛍光抗体法によるアッセイを薦めている。そこで、本研究では、非感染性のHIV粒子を産生する持続感染細胞株L-2を抗原として用いる蛍光抗体法によるアッセイの有用性について検討した。インドで集められた計2104血清サンプル（HIV感染の各種ハイリスクグループ）を用いて、NACOガイドラインに従って迅速診断キットでの判定を行うとともに、L-2細胞を用いた蛍光抗体法による判定も行った。その結果、陽性の212サンプルと陰性の1889サンプルは、両アッセイで同じ結果であった。残る3サンプル（0.14%）では、EIAでは陰性、蛍光抗体法では弱く陽性であった。一方、ウェスタンブロット法でも陰性（Gag/Pol蛋白への反応は陽性であったが、Env蛋白への反応が陰性）であった。しかし、これら3サンプルのいずれも、プラズマ中のウイルスRNAはRT-PCR法により検出された。このように、L-2細胞を用いた蛍光抗体法は、HIV感染の有無を調べる診断法として特異性および感度が高いことが明らかとなり、インドにおける確定診断法として考慮するに値することを見出した点において評価でき、学位の授与に値すると考えられる。