Immunofluorescence assay in India for confirmation of HIV-1 infection using a T-cell line infected with defective HIV-1

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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)が推奨するELISA迅速診断法が採用されている。確定診断においても、別の箇所に基づく迅速診断法が採用されている。しかし、アメリカでは、確定診断としてウェスタンプロット法もしくは重光抗体法によるアセッセイを推奨している。そこで、本研究では、非感染性のHLA-Ⅱ型粒子を産生する脊椎感染細胞株L-2を抗原として用いる蛍光抗体法によるアセッセイの有用性について検討した。インドで用いられるELISA迅速診断法（HIV感染の各種ベルテクスグループ）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L-2細胞と陰性の1059サンプルは、感度および特異性で一致した。L-2サンプル（0.14%)では、ELISAでは陰性、蛍光抗体法ではなく陽性であった。一方、ウェスタンプロット法では陽性、Gag/Pol組換え体への反応は陰性であった。しかし、これらの結果はいずれも、プラズマ中の中和抗体HIV-1-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ことが見直し出した点において評価でき、学会の授与に値すると考える。

【目的】

In India, the enzyme immunosassay (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 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 dengue-like 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 0–4 according to the intensity of fluorescence intensity in an approximately 25% of L-2 cells present in a monolayer of the HIV-infected cell line. IF and a nested reverse transcriptase polymerase chain reaction (RT-PCR) with primers targeting the C2–V3 fragment of the env gene, were performed on discrepant samples. An infectious molecular clone of Indian subtype C HIV-1, Indi-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/Pot, 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.