Diagnostic Methods

Presentation

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HIV-1 neutralization assay: a weak point of the TZM-bl reporter cell line

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The HeLa-derived TZM-bl reporter cell line (NIH AIDS Reagent program #8129, also called JC57BL-13) is widely used in laboratories for the estimation of the HIV/SIV titer and for testing sera neutralization potential. The cell line was engineered by amphotropic retroviral transduction to express CD4 and CCR5 and further on by transfection with two lentiviral vectors coding for the reporter genes - firefly luciferase and E. coli β-galactosidase under the HIV-1 LTR.

Here we provide evidences that TZM-bl cells express a recombinant protein - p47TZM, with internal part representing ectodomain of the transmembrane protein gp41 of HIV-1. The protein form aggregates in TZM-bl cells and can be isolated from the cell supernatant by high speed centrifugation. The p47TZM is well recognized by broadly neutralizing monoclonal antibody (Mab) targeting the gp41 membrane proximal external region (MPER) - 2F5 and to a significantly less degree by 4E10. The protein is likely encoded by mutated lentiviral transfer vector(s).

The effect of the p47TZM on HIV-1 neutralization by 2F5 and 4E10 Mabs was estimated on Jurkat and TZM-bl cells. For testing on Jurkat cells, 2F5 was mixed with normal human serum (nhs) and with cell-free supernatant from TZM-bl and 293T cells. The mixtures were incubated with HIV-1NL-4-3 for 1 h and loaded on cells. 48 h later the DNA from Jurkat cell was isolated and proviral load was measured by real-time PCR. It came out that cell-free supernatant from TZM-bl cells reduced the neutralization efficacy of 2F5, compared to that of 293T cells. Testing on TZM-bl cells was performed with both 2F5 and 4E10 Mabs, which were mixed with nhs, HIV-1NL-4-3 and cell-free concentrated supernatants from 293T and TZM-bl cells, respectively. Infection of cells was measured using Bright-Glo™ luciferase assay. Neutralization assays on TZM-bl cells demonstrated that supernatant from TZM-bl cells decrease neutralization of 2F5 by 15%-17%, while neutralization by 4E10 was not affected.

Conclusion: 1) A recombinant protein - p47TZM containing the gp41 ectodomain was found in TZM-bl cells. 2) Extracellular p47TZM may interfere with 2F5-like antibodies targeting the MPER (or another region of the gp41 ectodomain) and reduce their neutralization potential, thus increase the probability of false negative results.

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Potential Use of Recombinant Dengue Virus NS2A Protein for Specific Diagnosis of Dengue Virus Infection

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Background: Serological diagnosis of dengue virus (DENV) infection is impaired by cross-reactivity of antibodies against other flaviviruses in the currently used antibody tests. This may be due to the use of viral lysates in the traditional DENV antibody ELISAs. The aim of this study was to select a DENV protein with low amino acid sequence homology compared with proteins from other flaviviruses and to establish an antibody ELISA based on the recombinant protein.

Methods: Amino acid sequences of DENV proteins were aligned with sequences of the corresponding proteins from yellow fever, West Nile and Japanese encephalitis virus. DENV genes were obtained from DENV strains 1-4 by RT-PCR, cloned and expressed as fusion proteins in E. coli, and purified. Antigenicity was tested by ELISA and Western blot. Sera from the Sudan were examined by commercial ELISA and antibody positive and negative sera were used for NS2A ELISA feasibility testing.

Results: Among seven DENV proteins, the NS2A protein showed the lowest sequence homology with other flaviviruses (18.3-21.8 %). Sera that were positive in the DENV lysate ELISA showed strong reactivity with the NS2A protein. Negative sera and sera from individuals with West Nile virus infection did not react with the recombinant protein.

Conclusion: Infection with DENV induces a vigorous antibody response against the NS2A protein suggesting that the protein can replace viral lysate as antigen in serological diagnostics. As the protein has low sequence homology with NS2A proteins from other flaviviruses, antibody testing based on the DENV NS2A protein could increase the specificity of serological testing for DENV infection.