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Heat Shock Induces HIV-1 Replication in Chronically Infected Promyelocyte Cell Line OM10.1 : Implication of a New Anti- HIV-1 Assay In Vitro.

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We have developed a simple and reliable assay system for evaluating compounds that can prevent the activation of latent HIV-1. This system allow us to provide a new approach to the chemotherapy of AIDS. Although the inhibitory effects of compounds on activation of HIV-1 have been examined in several chronically infected cell lines treated with cytokines such as tumor necrosis factor α (TNF-α), we have found that heat shock exposure to OM10.1 cells (promyelocyte cell line latently infected with HIV-1) leads to high level production of HIV-1 without addition of any cytokines. The mechanism of activation has also been analyzed by using various inhibitors. The cells were incubated at 42 °C for 2 h and then at 37 °C for 36 h. Induction of HIV-1 replication was assessed by p24 antigen level and reverse transcriptase (RT) activity in the culture supernatants. The expression of HIV-1 antigens in OM10.1 cells was also examined. Although the level of TNF-α in the culture supernatants appeared to be below the sensitivity of a TNF-α detection ELISA system, addition of anti-TNF-α antibody in the culture medium partially suppressed the production of HIV-1 in the heat shock-treated OM10.1 cells. Furthermore, staurosporine (PKC inhibitor), pentoxifillin (NF-κB inhibitor), and Ro5-3335 (HIV-1 Tat inhibitor) could inhibit significantly the expression of HIV-1 antigens in the cells. These results suggest that several factors related to cellular signal transduction pathways are involved in the mechanism of activation. Thus, the present system may be useful to study the pathogenesis of HIV-1 infections and to discover a new class of effective anti-HIV-1 agents.

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High Throughput Screening for Small Molecule Inhibitors of the HIV Rev-RRE Interaction.

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It has been shown that small molecules can recognize both structural and primary sequence elements of RNA and bind to RNA with high specificity and affinity. Biological activity of small molecule RNA ligands has been demonstrated with neomycin B. The aminoglycoside neomycin B binds specifically to the HIV Rev-responsive element (RRE), blocking the interaction between the Rev protein and the RRE. These observations underscore the potential of RNA as a target for small molecule drugs. To identify new classes of inhibitors of the Rev-RRE interaction, we have developed a high throughput nitrocellulose filter binding assay. We have defined conditions under which Rev binds with high specificity to RRE RNA and non-specific interactions are undetectable. Our assay is robust and shows good reproducibility, permitting unambiguous identification of hit compounds. Screen design, results and further characterization of identified hit compounds will be presented.