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Intracellular Immunization against HIV-1 by Poly-TAR RNA Elements

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The HIV-1 *tat* protein activates HIV-1 gene expression primarily at transcriptional level and is essential for virus production. In addition, *tat* exhibits biological effects which may contribute to the pathogenesis of HIV infection; therefore interference with *tat* function is therapeutically desirable. Our research goal is to develop protective genes for intracellular immunization. The approach we have been using to suppress HIV-1 gene expression is based on controlled overexpression of multimerized TAR RNA to sequester one or more component involved in the *tat* response. Since *tat* has no cellular analog, a modified HIV-LTR was used as an inducible promoter to drive the inhibitory gene product of viral expression. Cotransfection of an LTR controlled poly-TAR plasmid with LTR-tat and LTR-CAT plasmids inhibits the level of the reporter gene activity (CAT) as much as 97%. The downregulation of HIV-I gene expression observed is dependent on the quantity of transfected poly-TAR as well as the number of tandem TAR repeats expressed per unit transcript. Similar constructs lacking either LTR upstream sequences or TAR sequence have no significant effect, suggesting that the competitive effect is mediated at the RNA level. To ensure the high efficiency gene transfer into the target cells, LTR-50TAR was inserted into the 3' LTR of the DC (double copy) retroviral vector (gift from E Gilboa) and the resultant plasmid (DC-LTR50TAR) was converted into the corresponding virus using a murine amphotropic packaging line. T cell lines chronically infected with HIV-1 were superinfected with retrovirus containing DC-LTR50TAR or with retroviral vector alone as control. After G418 selection, HIV-1 expression was analyzed. Uninfected T cell-lines were also infected with retovirus containing both constructs and selected for G418. Preliminary results indicated significant inhibition of HIV-1 production.

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Human immunodeficiency virus (HIV) replication: Modulation by cellular levels of cyclic adenosine monophosphate (cAMP). M. A. Nokta and R. B. Pollard. Division of Infectious Diseases, The University of Texas Medical Branch, Galveston, Texas USA.

HIV infection is associated with qualitative and functional deficiencies. Previously we reported that in vitro infection of CD4 positive cells with HIV was associated with sustained elevation of cAMP and cGMP. In this report the role of cAMP on HIV replication in MT-4 cells was investigated. MT-4 cells were infected with HIV (strain 3b), in the presence or absence of agents that increase intracellular levels of cAMP, through different mechanisms. At selected times post infection, HIV replication was measured by reverse transcriptase activity or HIV P24 Ag in culture supernatants. Forskolin (FK, an activator of adenylate cyclase 1-100 uM), Isobutylmethylxanthene (IBMX, a phosphodiesterase inhibitor, thus indirectly increasing intracellular levels of cAMP, 30-100 uM) and dibuteryl (db) cAMP (0.1-10 uM) enhanced HIV replication, in a dose dependent manner. FK, IBMX and db cAMP enhanced HIV replication by 2 to 10 fold, 4 to 7 fold, and 2 to 6 fold respectively. Intracellular levels of cAMP were measured simultaneously by radioimmunoassay and were also enhanced. Since cAMP exerts its catalytic effects through activation of protein kinase (PK) A, the effect of H-8 (a specific inhibitor of the cAMP dependent PK A) on HIV replication was simultaneously examined. H8 at doses of 0.1 to 10 um inhibited HIV replication by 25 to 99.9%. Moreover H8 inhibited HIV replication in peripheral blood mononuclear cells by more than 90%. In conclusion HIV replication appears to be a cAMP dependent event, and that PK A could possibly be a target for development of anti-HIV therapies.