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Phosphorylation and Subcellular Distribution of HIV-1 MA Protein

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The prototypic lentivirus HIV-1, in contrast to oncoretroviruses such as MLV, has the ability to integrate within cells which are non-dividing. This property of HIV-1 is in part accounted for by the karyophilic property of the viral preintegration complex which localizes in the nucleus after virus infection. We have demonstrated previously that HIV-1 *gag* MA protein is rapidly transported to nuclei of virus infected cells. The protein is associated with viral preintegration complexes and is, in part, responsible for its karyophilic properties and its transport to the nuclei of non-dividing cells. We have examined modification of HIV-1 MA at early steps after acute infection in order to further delineate its function. Our results indicate that MA within the context of the viral preintegration complex is rapidly phosphorylated, a modification which coincides with its localization in the nucleus. This localization occurs despite the presence of a myristoylation modification on the protein: one which apparently is important for plasma membrane targeting during virus assembly. Phosphoamino acid analysis of MA from acutely infected cells identified phosphoserine. It is proposed that inhibition of MA phosphorylation will interrupt function of the viral preintegration complex and may inhibit virus replication.

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Replication of HIV-1 and HSV-1 in Macrophages: Comparative Activity of Acyclic Nucleoside Phosphonates in the Presence or Absence of Cytokines Enhancers of Virus Replication

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We investigated the ability of cytokines and acyclic nucleoside phosphonates to affect the replication of HIV-1 and herpes simplex type 1 (HSV-1) in primary monocyte/macrophages (M/M). Both granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage-colony stimulating factor (M-CSF) significantly enhanced the replication of HIV in M/M, GM-CSF being 3-10 fold more potent than M-CSF ($p<0.05$). Also HSV-1 easily replicates in M/M, with a clear cut cytopathic effect within 3-5 days after the virus challenge. Both cytopathic effect and virus replication (the latter measured as virus titer) were substantially enhanced by GM-CSF and M-CSF ($p<0.01$ in both cases compared to controls). In contrast with HIV-1, HSV-1 replication is enhanced by M-CSF 10-50 fold more than GM-CSF ($p<0.01$). The antiviral activity of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) is substantially greater in M/M for both HIV and HSV-1 (EC50 being 0.02 uM and <0.3 uM respectively) when compared to its antiviral activity in lymphocytes (EC50 about 3uM for HIV-1) and in fibroblastoid cells (EC50 about 2 uM for HSV-1). This antiviral effect is related both to the increased levels of phosphorylation of PMEA to its active form PMEA-diphosphate (4 fold greater in M/M than in T-lymphocytes), and to a contemporaneous decrease of the endogenous competing dATP in M/M in respect with replicating cells such as T-cells and fibroblastoid cells. Conceivably due to the virus enhancement, GM-CSF and M-CSF substantially decreased the antiviral activity of PMEA against HIV-1 and HSV-1 in M/M. In either case, however, complete inhibition of the replication of HIV and HSV-1 was achieved at concentrations far below those toxic for M/M. The reduction of the antiviral activity of PMEA is at least in part explained by the cytokine-induced increase of replication of both viruses. By contrast, the levels of PMEA-diphosphate are similar in both cytokine-treated and not-treated M/M. Since these cytokines are present in the body at concentrations similar to those used in these experiments, the results achieved in vitro may somewhat be representative of those occurring in patients. Nevertheless, overall results suggest that PMEA is a potent inhibitor of HIV-1 and HSV-1 in M/M, cells representing a reservoir in vivo for both viruses.