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Differential Expression of Host Cellular Factors upon HIV-1 Reactivation

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The development of antiretroviral therapy (ART) has achieved almost complete suppression of HIV-1 replication in infected individuals (plasma viral load <50 copies/mL). However, integrated HIV-1 remains hidden in long-lived reservoir cells, such as latently infected $CD4^+$ T lymphocytes and monocyte/macrophages. As a consequence, infected individuals receiving ART often experience the rebound of plasma viral load at the time of discontinuation of ART. Since latent HIV-1 can be reactivated from the reservoir cells leading to viral dissemination and disease progression, a clear understanding of the mechanisms of viral latency and reactivation is a key to designing novel therapeutic agents and achieving eradication of these viral reservoirs. Recent genomic studies have identified several cellular factors associated with viral latency, suggesting that a subset of host-virus interactions is triggered during HIV-1 reactivation. In order to identify cellular factors differentially expressed upon HIV-1 reactivation, a comprehensive and wide genome expression analysis was conducted using a model of promyelocytic cells latently infected with HIV-1. Microarray expression profiles were analyzed at different time points after stimulation for inducing active viral replication in the latently infected cells. In this study, a subset of genes was differentially expressed in the stimulated cells when compared to the unstimulated cells and their uninfected parental cells (332 genes upregulated vs. 102 genes downregulated). Significant upregulation was observed for genes related to signal transduction, immune response, G-protein coupled receptor protein signaling pathway, cell–cell signaling, ion transport and cell adhesion. Our results highlight the role of a subset of host factors involved in viral latency, indicating their potential use as novel cellular targets for inhibition of HIV-1 replication and inhibitor design. Undergoing studies, including knockdown of the genes and evaluation in other cell lines and primary T lymphocytes, are aimed to elucidate the role of selected host factors in viral replication.

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Recycling of HIV Particles is Required for Infection by Endocytosed Virus after Cell to Cell Transfer

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Although it has long been assumed that HIV entry occurs by direct fusion between virus and cellular membranes at the cell surface, recent alternative evidences suggests that HIV particles may fuse with endosomal membranes, being this route the only productive entry pathway. Thus, the role of endocytosis in HIV replication and whether or not endocytic virus transfer represents an escape mechanism from the immune system or therapeutic agents remains highly controversial. Parallel cocultures, in the presence of different entry inhibitors, between HIV-1-infected MOLT NL4-3 effector cells and previously stimulated or non-stimulated primary CD4+ lymphocytes, induced the transmission of high amounts of HIV-antigen (around 20% of p24 positive T cells in all conditions, except for Leu3a which inhibited 75% of transfer in both cases). After coculture, purified trypsin-treated target cells were left in culture in different medium conditions, designed specifically to evaluate the infectious pathway taken by the captured HIV particles. Purified, trypsin-treated target cells became infected if left in culture medium. Conversely, cells did not become infected if cultured in the presence of mAb IgGb12, an agent that blocks virus attachment to CD4 (6 and 1.3-fold increase in supernatant p24 production in non- and stimulated CD4+ T cells, respectively). Importantly, similar results were obtained if the transfer of HIV particles occurred in the presence of the HIV entry inhibitors BMS155 or AMD3100. Our results suggest that HIV could not infect cells through direct fusion from within endosomal compartments and required recycling to the cell surface to initiate a productive infection. Consequently, the HIV-transmission process is presented here as an itinerant virus reservoir, capable to generate trans-infection after the release of the HIV particles to the extracellular environment. Since cell-to-cell HIV transfer is considered one of the most efficient mechanisms of HIV spread, new insights in the mechanism of HIV transfer allows the identification and characterization of novel potential targets for HIV anti-retroviral therapy.

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Inhibition of Influenza Virus-induced NF-kB and ERK Activation can Simultaneously Reduce Both, Virus Titres and Cytokine Expression *In Vitro* and *In Vivo*

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Influenza virus (IV) infection can cause severe pneumonia and lead to acute respiratory distress syndrome and death. So far therapeutic actions are limited to vaccines and a few anti-viral drugs. These drugs target functions of the virus itself thereby selecting drug-resistant variants. During their replication IV activate ERK and the transcription factor NF-kB. Both result in pro-viral as well as anti-viral effects by promoting nuclear export of the viral genome to be packaged into progeny virions and by inducing expression of pro-inflammatory host defence factors. Apart from tissue damage caused by the lytic replication of the virus, an imbalanced overproduction of anti-viral cytokines ("cytokine burst") can lead to severe lung damage as observed in human infections with highly pathogenic avian IV (HPAIV) of the H5N1-type. Recently we have shown that inhibition of NF-kB activity reduces virus titre *in vitro* and *in vivo*. As a proof of principle we now analyzed whether it is possible to target both aforementioned pathways with specific inhibitors in order to reduce virus titres as well as virus-induced cytokines, simultaneously. We show that reduced activity of both pathways by specific inhibitors indeed leads to decreased virus titres and cytokine expression. This was not only true *in vitro* for permanent A549 cells or primary mouse alveolar epithelial cells infected with human IV or HPAIV, but also *in vivo* in IV-infected mice. Our results hereby demonstrate for the first time *in vitro* and *in vivo* that inhibition of ERK and NF-kB activity can be used to reduce virus titres and modulate pro-inflammatory cytokine expression, concurrently. This could provide new rationales of future therapeutic strategies to treat influenza virus pneumonia.

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