R5 HIV-1 strains were cultured in lymphoid cells expressing high levels of CXCR4 and low levels of CCR5 in the absence or presence of agents targeting CXCR4 (AMD3100), CCR5 (Tak-779, mAb 2D7 and RANTES) and the reverse transcriptase (AZT). Cell cultures were kept for up to 200 days. Syncytium formation and virus replication (p24 antigen in the supernatant) were recorded weekly. Viral coreceptor use was evaluated in MT-2 cells and U87-CD4+CCR5+/CXCR4+ cells at different times. Proviral DNA of selected passages was used to identify amino acid changes in env. Virus replication was reminiscent of slow replicating, R5 phenotype but could be blocked by Tak-779, RANTES or mAb 2D7. One of the six strains was resistant to AZT. In the absence of drug pressure, three out six strains used were able to switch from the R5 to the X4 phenotype and showed increased replication rate. Coreceptor switch could be delayed by AZT or Tak-779 in all three strains. However, under similar drug-pressure, outgrowth of virus could be detected faster in the presence of CCR5 inhibitors but not in the presence of AZT and was concomitant to its ability to use CXCR4. Coreceptor switch was noticed earlier in the presence of AZT than Tak-779 in the culture of the AZT-resistant virus. Conversely, treatment with AMD3100 prevented the emergence of CXCR4-using virus. In conclusion, under our experimental conditions, a lower replication rate (i.e. AZT or Tak-779 versus no drug treatment) slows coreceptor switch. However, under similar replication conditions (i.e. AZT versus Tak-779) CCR5 drug pressure may induce a faster emergence of CXCR4-using HIV. A cell culture model of the evolution of HIV-1 coreceptor use may be relevant to assess the propensity of clinical isolates to develop drug resistance through a change in virus coreceptor.

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Induction of IL-6 and IL-8 by siRNAs Targeting HIV Coreceptor CCR5

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The HIV-1 coreceptor CCR5 has been thought a relevant target for small interfering RNA (siRNA)-based therapeutics. However, recent findings suggest that siRNA can stimulate innate cytokine responses in mammals. All siRNA agents tested were able to down-regulate the expression of CCR5 albeit with different efficiency (51-74% downregulation), block HIV induced syncytia formation between HIV-1 BaL-infected and uninfected CD4+ cells or block single round HIV-1 infection as measured by a luciferase reporter assay (46-83% inhibition). Conversely, siRNA directed against CCR5 did not affect replication of a VSV pseudotyped virus, suggesting that inhibition of HIV replication was specific to CCR5 down-regulation. However, 2 of 4 siRNA tested were able to induce the production of interleukin-6 (6-fold induction) and interleukin-8 (9-fold induction) but no IFN-(, IFN-(, IFN-(, TNF-(, MCP-1, MIP-1(, MIP-1(, RANTES, IL-1(, IL-10 or IL-12p70 cytokine induction was noted. In the absence of detectable IFN-(, IL-6 or IL-8 may represent markers of non-specific effect triggered by siRNA.

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Identification and Characterization of a Novel, Potent HCV Helicase Inhibitor

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Hepatitis C virus (HCV) is the leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide. Current interferon-based standard of care has demonstrated limited success and is associated with undesirable side effects. Significant efforts have been directed towards the discovery and development of effective therapeutics to treat HCV infections and patients relapsing from interferon therapy. We undertook the task to screen a compound library for anti-HCV activity using the subgenomic HCV replicon-based reporter system. Trioxsalen was identified as a potent HCV inhibitor. Trioxsalen belongs to the group of medicines called psoralens, and is an FDA approved drug used along with ultraviolet light to treat vitiligo. The compound suppressed replication of the HCV replicon in a dose-dependent manner with a EC_{50} value of 1.8 μM. Interestingly, trioxsalen had no antiviral activity against both BVDV and YFV, members of flaviviruses related to HCV and two trioxsalen analogs, 5-methoxypsoralen and 8-methoxypsoralen, did not display any inhibition in the HCV replicon system. In order to determine the mechanism of action of trioxsalen, the compound was examined for inhibition of the HCV NS5B polymerase, the NS3 helicase and the NS3/4A proteinase. The results demonstrated that trioxsalen did not exhibit appreciable inhibitory effects on the NS5B polymerase activity and the NS3/4A proteinase activity; however, the compound acted as a potent inhibitor against the helicase activity by inhibiting the unwinding process. As the helicase is crucial for the HCV life cycle, trioxsalen may represent a novel class of therapeutics to treat HCV infections.

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