Retroviral vectors for gene therapy are designed to minimize the occurrence of replication-competent retrovirus (RCR); nonetheless, it is possible that a vector-derived RCR could establish an infection in a patient. Since the efficacy of antiretroviral agents can be impacted by interactions between virus, host cell, and drug, five commonly used antiretroviral drugs were evaluated for their abilities to inhibit the replication of a murine leukemia virus (MLV)-derived RCR in human cells. The results obtained indicate that the combination of nucleoside analogs zidovudine and dideoxyinosine with the protease inhibitor indinavir effectively inhibits MLV-derived RCR replication in three human cell lines. In addition, MLV-derived RCR was found to be inherently resistant to the nucleoside analogs lamivudine and stavudine, suggesting that mutations conferring resistance to nucleoside analogs in human immunodeficiency virus type 1 have the same effect even in an alternative viral backbone.
the most commonly used clinical treatment for HIV infection (26).

The studies described above demonstrate that three clinically relevant antiretroviral agents are effective in inhibiting the replication of murine RCR in 293 cells. To ensure that the drugs identified were also active in other human cell types, these drugs were tested alone and in combination in 293 cells as well as two human cell lines of lymphoid origin, Jurkat and H9 cells. In order to be able to detect decreases in viral infectivity for the combination treatments, an intermediate dose of drug was chosen for the combination therapies tested; each drug was tested singly in the same experiment so that any additive effects of the combination treatments could clearly be assessed. H9 and Jurkat cells were infected and passaged under the same conditions as those described previously for 293 cells. For increased sensitivity of detection, infectivity was measured by a quantitative PCR assay for the presence of viral envelope sequences. This RCR PCR assay amplifies a 712-bp region of the envelope sequences contained in pPAM3, the packaging sequence present in PA317 cells (21). The primers used for amplification were 5'-TTGTCCACCAAGTGCTCTTCA AAT-3' and 5'-GGCTCAGTACTATTAAGCTTC-3'. Amplification products were analyzed by gel electrophoresis and Southern blotting and were analyzed on a Storm imaging system (Molecular Dynamics, Sunnyvale, Calif.). The results of these experiments are shown in Fig. 3.

The efficacy of AZT, ddl, and indinavir alone varied between cell types. For example, H9 cells were more sensitive to both nucleoside analogs than either 293 or Jurkat cells and appeared to be extremely sensitive to ddl (96.6% inhibited at 40 \(\mu M\)). In contrast, infectivity in Jurkat cells appeared to be unaffected by 0.05 \(\mu M\) AZT and was only slightly affected by 40 \(\mu M\) ddl. These results support previous studies demonstrating that antiretroviral efficacy can vary between cell types, even in an in vitro assay system in which identical virus and conditions were used to establish infection. However, in all three cell lines, the combinations of two and three drugs were more effective than any single drug. In the most resistant cell line assayed, Jurkat, the AZT-ddl combination inhibited approximately 24% and the addition of the protease inhibitor indinavir further reduced activity to 36% of the untreated control cultures. In H9 and 293 cells, infectivity in the triple combination-treated cells was reduced to 1.1 and 11.3% of that of the untreated control cells, respectively. The approximate 50% inhibitory concentrations (IC\(_{50}\)) of AZT and indinavir in 293 cells were 0.08 and 3.0 \(\mu M\), respectively, which are similar to IC\(_{50}\)s reported in in vitro studies against HIV-1 and well within the achievable concentrations of these drugs in vivo (maximum concentration of drug in serum [\(C_{\text{max}}\) for AZT, 3.4 \(\mu M\); \(C_{\text{max}}\) for indinavir, 12.6 \(\mu M\)] (4-6, 9). While the IC\(_{50}\) for ddl determined in 293 cells (approximately 40 \(\mu M\)) exceeds the \(C_{\text{max}}\) in vivo (8.2 \(\mu M\)), the increased sensitivity of H9 cells to this drug suggests that ddl may have efficacy against some cell types in vivo. These results demonstrate that the use of the AZT-ddl-indinavir combination therapy yields a good inhibition of murine RCR replication in three different human cell types.

Although distribution studies and patient monitoring indicate that retroviral vectors can potentially be found in a number of tissues, it is not clear what cell type, if any, might be particularly susceptible to RCR infection. In the one study in which murine RCR infection was established in immunosuppressed primates (7), three of eight monkeys died of T-cell lymphoma; the surviving monkeys have remained positive for viral sequences at very low levels in peripheral blood lymphocyte samples. It is worth noting that monkeys that survived the infection developed an antibody response to the RCR but

FIG. 1. Efficacies of antiretroviral drugs against murine RCR replication in 293 cells. 293 cells were infected with G1Na.40 RCR; drugs were added simultaneously with virus to block the establishment and spread of infection. Cultures were passaged in the presence of drug at 4 and 7 days postinfection, and the medium collected 10 days postinfection was precipitated with polyethylene glycol for RT assay. The doses tested were as follows: AZT, 0.01 to 5 \(\mu M\); ddl, 0.1 to 100 \(\mu M\); 3TC, 0.1 to 50 \(\mu M\); indinavir, 0.1 to 10 \(\mu M\). The relative efficacies of the drugs tested may be arranged as follows: AZT > indinavir > ddl > d4T (data not shown), 3TC.

FIG. 2. Alignment of MLV and HIV-1 RT sequences. The sequences of Moloney MLV (Mo-MLV) and HIV-1 RT were aligned in regions flanking mutations known to confer resistance to one or more drugs. The murine RCR used in these studies was sequenced and found to be identical to the Moloney sequence in these regions (data not shown). (A) 3TC resistance mutation occurs at amino acid 184 of HIV (shaded box); the sequence of the Moloney RT in this region is identical to that of 3TC-resistant HIV-1. (B) d4T resistance mutation occurs at amino acid 75 of HIV (shaded box). The sequence of MLV RT in the homologous position differs from wild-type HIV at several residues in this region.

FIG. 3. Antiretroviral efficacy assays. Combinations of RT inhibitors and protease inhibitors are frequently used in clinical and research settings. In these experiments, the IC\(_{50}\)s of the drugs tested were as follows: AZT, 0.01 to 5 \(\mu M\); d4T, 0.01 to 100 \(\mu M\); 3TC, 0.1 to 50 \(\mu M\); indinavir, 0.1 to 10 \(\mu M\). The relative efficacies of the drugs tested may be arranged as follows: AZT > indinavir > ddl > d4T (data not shown), 3TC.

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those that died did not. Individual animal variability in the degree of immune system suppression and/or immune system recovery following transplantation may be responsible for differences in RCR pathogenesis. An early intervention with the combination of antiretroviral drugs identified in this study might limit the viral load in multiple cell types and allow sufficient time for immune system recovery and long-term survival.

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