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A NEW CLASS OF ANTI-HIV-1 OLIGONUCLEOTIDE TARGETED TO THE POLYPURINE TRACT OF VIRAL RNA

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ABSTRACT

The PPT is highly conserved among the known HIV-1 strains, and is a possible target for triplex formation. We show triple-helix formation by a two-strand-system (FTFOs, DsDGloopT5-37) targeted to the polypurine tract (PPT) of HIV-1. In HIV-1 infected MOLT-4 cells, the FTFOs containing phosphorothioate groups at the antisense strand and guanosine rich parts within the third Hoogsteen base pairing sequence inhibit the replication of HIV-1 more effectively than the antisense phosphorothioate oligonucleotides indicating sequence-specific inhibition of HIV-1 replication for 62 days. However, AZT, treated cells expressed high levels of p 24 products after 46 days.

Replication of HIV-1 proceeds by means of the reverse transcriptase (RT), which catalyzes the conversion of the single-stranded viral RNA genome into double-stranded DNA and allows integration into the cellular genome (1-3). This process involves multiple steps. The RNase H activity of the RT catalyzes the hydrolysis of the viral RNA from an RNA/DNA hybrid molecule (3). A polypurine tract (PPT) consisting of 16 nt, which is resistant to RNase H cleavage, serves as

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a primer for plus-strand DNA synthesis by the DNA-directed DNA polymerase activity of the RT. The PPT is a highly conserved region adjacent to the 3'-end (U3) of the viral RNA, and it has an essential function during reverse transcription (4). When the homopolymeric PPT functions as a target sequence, it can hybridize with antisense oligonucleotides as well as triple-helix-forming oligonucleotides. Previously, we observed that the FTFOs containing phosphorothioate groups at the antisense strand and guanosine rich parts within the third Hoogsteen base-pairing strand showed high anti-HIV-1 activity in HIV-1 infected MOLT-4 cells (5).

In this paper, we describe the anti-HIV-1 activities of the FTFOs and AZT in a long-term experiment (62 days).

RESULTS AND DISCUSSION

In order to clarify the anti-HIV activities of the FTFOs, we examined the long-term effects of FTFOs treatment in HIV-1 infected MOLT-4 cells. The MOLT-4 cells were incubated with HTLV-III_B for 2 h to allow absorption. The cells were then washed to remove the virus from the medium, and the modified FTFOs were added with fresh medium. After 2 days, new medium supplemented with the oligonucleotides was added. The virus production in the culture supernatant was monitored by the HIV-1 p 24 antigen assay (Fig. 1). The control-infected cells (no oligomer added) exhibited maximal HIV-1 replication at 18 days. However, in the cells treated with the DsDGloopT5-37 (1 μ M), p 24 expression was inhibited by 100%, as compared to the untreated control. Interestingly, the short FTFOs, DsDGloopT5-29, inhibited virus replication by 100%, as compared to the untreated control at 58 days. On the other hand, AZT inhibited virus replication by 100%, as compared to the untreated control at 30 days, but the treated cells expressed high levels of p 24 products after 46 days. The ineffectiveness of AZT may cause AZT-resistant HIV strains.

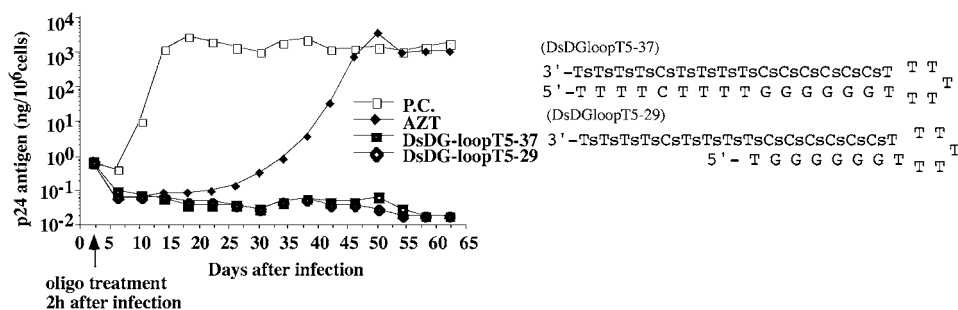


Figure 1. Antiviral activity of the FTFOs at 1 μ M. After 2 h, the virus was removed from MOLT-4 cells newly infected with HTLV-III_B, and the cells were treated with the synthetic oligonucleotides. The second treatment was performed 2 days later. Supernatants were collected and p 24 expression was determined.



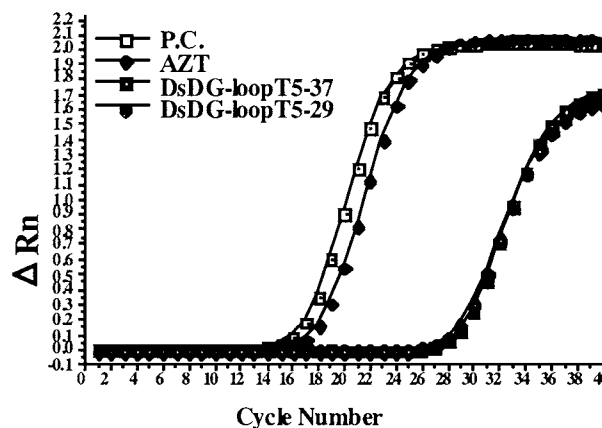


Figure 2. Real-time PCR quantification of HIV-1 RNA. MOLT-4 cells were infected with HTLV-IIIIB (MOI = 0.01) and were treated with 1 μ M of the FTFOs.

After 62 days of infection, we detected the viral RNA by a spectrofluorometric thermal cycler (ABI PRISM 770; PE Applied Biosystems). The targets of the primer set were located after the PPT region of HIV-1. The primers were chosen so that they would not interact with each other and their target sequences would be unique to HIV-1. The probe sequence of each molecular beacon was chosen so that it would hybridize to a sequence within its target amplicon. Real-time PCR was performed with a Taqman EZ RT-PCR kit (PE Applied Biosystems), so that only RNA was amplified. As a result, the p 24 antigen assay sample treated with DsDGloopT5-37 had a reduced amounts of RT-PCR products (Fig. 2). The greatest inhibitory effects on HIV-1 replication were detected with the DsDGloopT5-37 at the 1 μ M concentration.

In conclusion, in HIV-1 infected MT-4 cells, the FTFOs containing the phosphorothioate groups at the antisense sequence sites and guanosine rich parts within the third Hoogsteen base-pairing strand inhibit the replication of HIV-1 more effectively than the anti-S-ODNs and AZT indicating sequence-specific inhibition of HIV-1 replication.

EXPERIMENTAL

Oligonucleotides. The oligonucleotides were purchased from Genset Oligos Co.

Anti-HIV assay. The CD4⁺ T-cell line, MOLT-4 (3×10^5 ml⁻¹), was infected with HTLV- IIIB at a MOI of 0.01. After a 2 h infection, the cells were washed and treated with the synthetic oligonucleotides at a 1 μ M concentration in the culture medium. After 2 days the medium was removed and fresh medium containing the oligonucleotides at a 1 μ M concentration was added. Virus replication was monitored at the cellular level by syncytia formation and in the culture supernatants by

the p 24 antigen using a chemiluminescence enzyme immunoassay (CLEIA, 2 step sandwich method; Fujirebio) (34). At the time points indicated, an aliquot of the culture supernatant was removed for p 24 antigen analysis and was replaced by fresh medium. Every 4 days, viable cells were counted and passed at 3×10^5 cells per ml.

Real-time PCR analysis. Infections were performed as previously described. Total cellular RNA was extracted by the RNeasy Total RNA System (QIAGEN). The Taqman EZ RT-PCR kit (PE Applied Biosystems) was then used to synthesize the cDNA. The sequence of the molecular beacon is 5'-FAM-AGGTTTGACAG-CCGCC- TAGCATTTTCAT-TAMRA-3' (nucleotides 9345 to 9371), where 6-carboxyfluorescein (FAM) serves as the reporter fluorochrome and tetramethylrhodamine (TAMRA) serves as the quencher. One cycle of an initial step (50°C for 2 min), an RT step (60°C for 30 min), and a denaturation step (95°C for 5 min) was followed by 40 cycles of amplification (94°C for 20 s. and 62°C for 1 min). PCR was carried out in a spectrofluorometric thermal cycler (ABI PRISM 7700; PE Applied Biosystems) that monitors changes in the fluorescence spectrum of each reaction tube during the annealing phase while simultaneously carrying out the programmed temperature cycles. The PCR primer pair used to amplify specifically amplify after the PPT region was 5'-ACCAGCTTGTTACACCCTGTGA-3' (nucleotides 9279 to 9300) and 5'-AAGTACTCCGGATGCAGCTCTC-3' (nucleotides 9403 to 9382). Real-time PCR was also performed with the primers and the probe provided with the Taqman β -actin Control Reagents (PE Applied Biosystems) to normalize each of the MOLT-4 cell RNAs.

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REFERENCES

1. Baltimore, D. *Nature* **1970**, 226, 1209–1213.
2. Temin, H.M.; Mizutani, S. *Nature* **1970**, 226, 1211–1213.
3. Moelling, K.; Bolognesi, D.P.; Bauer, H.; Busen, W.; Plassmann, H.W.; Hausen, P. *Nature New Biol.* **1971**, 23, 240–243.
4. Charneau, P.; Alizon, M.; Clavel, F. *J. Virol.* **1992**, 60, 2814–2820.
5. Hiratou, T.; Tsukahara, T.; Miyano-Kurosaki, N.; Tsukahara, S.; Takai, K.; Yamamoto, N.; Takaku, H. *FEBS Lett.* **1999**, 456, 186–190.



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