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Inhibition of Replication of Drug-Resistant HIV Type 1 Isolates by Polypurine Tract-Specific Oligodeoxynucleotide TFO A

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ABSTRACT

A 54-base-long oligodeoxynucleotide (ODN) termed *triple helix-forming oligonucleotide A (TFO A)*, designed against the 3′-polypurine tract (PPT) of the human immunodeficiency virus type 1 (HIV-1), exhibits long-term efficacy in antiretroviral treatment. Viral replication of strains propagated in this laboratory as well as primary patient isolates are inhibited by TFO A, whereas ODNs with a randomized sequence but identical base composition show no effect. TFO A inhibits proviral DNA synthesis. To learn more about the molecular mechanism of function of TFO A, three HIV-1 isolates whose reverse transcriptase (RT) exhibits resistance against RT inhibitors were analyzed. They exhibit resistance against azidothymidine, dideoxyinosine, deoxythiacytidine, and the nonnucleoside inhibitor nevirapine. HIV-1 replication in TFO A-treated T cell cultures was assessed by monitoring p24 viral core antigen production and syncytium formation. No p24 antigen or syncytia were detected for up to 30 days when cells that had been infected with wild-type virus received TFO A. Similarly, replication of all three mutant HIV-1 strains was completely inhibited by TFO A treatment during the whole duration of the culturing period. No viral breakthrough was detectable. These results indicate that TFO A interferes with functions of the replicative cycle distinct from polymerization by the RT.

INTRODUCTION

We have reported the use of an oligodeoxynucleotide (ODN) specific for the 3′ polypurine tract (PPT), which resides in the coding region of the *nef* gene adjacent to the unique region at the 3′ end (U3). A second PPT is found in the *pol* gene in the coding region of the integrase gene. Both PPT sequences are identical in the HIV-1 genome. The PPT consists of 16 consecutive purines and an extended 3′-PPT contains 2 nonpurine bases and, in addition, 7 purines, thus comprising altogether 25 nucleotides. The PPT is extremely well conserved in evolution and can be found in most human and primate immunodeficiency viruses. Mutations in this sequence are rarely found among HIV-1 strains, presumably because this sequence constitutes the recognition site of the reverse transcriptase (RT) to initiate plus-strand DNA synthesis after an RNase H cleavage step to expose a 3′-hydroxyl group at the 3′ end of the PPT sequence.[1–3] The seven purine nucleotides of the extended PPT adjacent to the U3 region are an essential recognition site for integration of the provirus.

The triple helix-forming oligonucleotide A (TFO A) was designed to form a triple helix to block replication of HIV-1[4] in acutely infected T cells.[5,6] It consists of two different domains, one allowing double-helix formation involving Watson–Crick base pairs with the target RNA, and the other allowing triple-helix formation involving Hoogsteen-type base triplets in the major groove of the stem of the polypurine tract.[7] To facilitate triple-helix formation the two strands were connected by a linker of four thymidines. The three terminal nucleotides as well as the T-linker were thioated in order to increase resistance against nucleases. The TFO A has been shown to allow triple-helix formation *in vitro* since two transition temperatures were observed in melting analysis. The structure formed at the PPT exhibits a two-step melting curve at 260 nm, with one transition at T_m 60°C corresponding to the dissociation of a triplex and another at 80°C corresponding to melting of the double strand.[6]

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Furthermore, we have demonstrated that HIV-1 replication in acutely infected T cells was completely inhibited with no breakthrough during the whole culturing period for up to 30 days. Syncytium formation and p24 synthesis were completely inhibited. In addition, we found that inhibition by triplex formation is by far superior to antisense-mediated antiviral effects. The biological effect of TFO A was shown to occur at a concentration of the ODN able to inhibit viral replication by 50% (IC_{50}) of 0.8 to 0.9 μM. The IC_{90} of TFO A was found to be equal to 1.3 μM. Further work demonstrated that TFO A was also able to suppress retroviral replication in peripheral blood mononuclear cells infected with three primary HIV-1 patient isolates taken at different stages of the disease.[8] Again, viral replication was completely inhibited. Amplification of DNA from TFO A-treated cultures failed to demonstrate the presence of viral DNA after HIV infection.[8] The molecular mechanism of TFO A in HIV replication is not yet known. Since the sequence of TFO A allows the formation of a self-complementary hairpin loop structure, we were considering the possibility that TFO A might directly interfere with the RT enzyme. One way to evaluate such an effect was to test drug-resistant RT mutants. So far, the antiviral function of TFOs has not been applied to drug-resistant HIV isolates.

Most of the drugs that are used for AIDS treatment block the viral RT activity and are DNA chain terminators that prevent the synthesis of viral DNA from the retroviral RNA genomic template. These nucleoside drugs are incorporated into the newly synthesized viral DNA strand in place of the usual deoxynucleotide, e.g., thymidine triphosphate (dTTP). Compounds such as the triphosphates of 3′-azido-3′-deoxythymidine (AZT; zidovudine, ZDV), and 2′,3′-dideoxyinosine (ddI, didanosine), 2′,3′-dideoxycytidine (ddC, zalcitabine), and the oxathiolane-cytosine analog 3TC (2′,3′-deoxythiacytidine) and its enantiomer 2′,3′-dideoxy-5′-fluoro-3′-thiacytidine (FTC),[9,10] can act as competitive inhibitors and as chain terminators leading to a complete arrest of proviral DNA synthesis. Resistance has also been demonstrated against some nonnucleoside inhibitors of the viral RT. Several researchers have reported that point mutations within the HIV *pol* gene, which encodes among other replicative enzymes the RT, are responsible for HIV-1 drug resistance. When viral isolates from patients on antiviral chemotherapy are investigated for the types of changes that occurred in the amino acid sequence of their RT, several mutations were frequently found. Studies have demonstrated that a mutation at amino acid residue 215 (T215Y) is present in the DNA of cocultures or viral RNA of asymptomatic patients on chronic AZT chemotherapy.[11] It is possible to prove the presence of resistance-conferring mutations by DNA amplification technology prior to the isolation of variant viruses that possess a drug-resistant phenotype. Investigations performed on peripheral blood mononuclear cells (PBMCs), plasma virus-derived RNA of patients, and cell culture-propagated viruses have documented mutations at amino acid positions M41L, D67N, K70R, T215Y, and K219Q in the RT molecule.[12,13]

In this article we test the replication of HIV-1 in the presence of TFOs, using viral isolates that exhibit resistance against some of the most widely used clinical drugs.

MATERIALS AND METHODS

Cells and viruses

For cell culture studies we used the HTLV-I-transformed T cell line C81-66/45, kindly provided by R.C. Gallo (Institute of Human Virology, Baltimore, MD).[14] Cells were grown in RPMI 1640 containing 20% heat-inactivated fetal calf serum (FCS), L-glutamine (2 mM), penicillin (50 U/ml), and streptomycin (50 μg/ml) (all purchased from GIBCO-BRL, Life Technologies, Basel, Switzerland). The following HIV-1 viral strains were used in this study: the AZT-resistant HIV-1_{RTMN} strain bears mutations at positions 41 and 215 (M41L and T215Y). The 3TC/FTC-resistant HIV-1_{184V} strain contains an RT mutation (M184V), and the multiple RT inhibitor-resistant HIV-1_{RTMDR1} isolate harbors the mutations M41L, L74V, V106A, and T215V and displays coresistance to AZT, ddI, and the nonnucleoside inhibitor nevirapine. The wild-type parental strain HIV-1_{HXB2} was also included in the tests and was a generous gift of R. C. Gallo. The mutant viral strains were obtained from the Medical Research Council (MRC) AIDS Reagent Project (Potters Bar, UK). We thank the original donors of the strains (B. Larder, P. Kellam, and S. Kemp).

Oligodeoxynucleotides

The following TFOs were used: phosphorothioated TFO A (5′-$T_sT_sT_s$TCTTTTGGGGGGTTTGGTTGGG$_sT_sT_sT_s$TCCCTTCCAGTCCCCCCTTTTCTT$_sT_sT_s$), consisting of a Watson–Crick base pair-forming sequence of 25 nucleotides directed against the extended PPT, a triple helix-forming sequence of 25 nucleotides, connected by a T linker of 4 nucleotides. The subscript(s) indicates the thioate modifications. A scrambled version of TFO A, used as control, was named TFO SC (5′-$T_sT_sT_s$GGGGGGTTCTTCCTCCTTTCCT$_sT_sT_s$ T_sTCGCCCGTCCGTTGCGTTGATTTT$_sT_sT_s$).

Assay for antiretroviral activity of TFOs using T cells

Experiments were performed as described.[6] Briefly, 2×10^5 C81-66/45 cells were infected with virus-containing supernatant that had been titrated for infectivity. In the case of the HIV-1_{IIIB} strain a low multiplicity of infection (MOI of 0.05) was used. Two hours postinfection cells were washed extensively and treated with the phosphorothioated oligodeoxynucleotides at 2 μM concentration. Twenty-four hours later supernatant medium was removed and fresh medium was added, again containing the ODNs at an identical concentration (second spike). After 4 to 5 days medium was replaced by fresh medium without ODNs for the remainder of the cultivation period. We had earlier used a lower concentration of the drug (1 μM) but we found that the inhibitory effect was more reproducible with the higher concentration of the oligonucleotide. HIV-1 mutant strains were obtained from the Medical Research Council, AIDS Reagent Project, National Institute for Biological Standards and Control (Potters Bar, UK). Three HIV-1 drug-resistant mutant strains that are descendants of the HIV-1_{HXB2} parental strain were used for this study (see Results).

Amplification of virus yield of HIV-1 mutant isolates

The host range of these viruses can be described as including neoplastic $CD4^+$ T cell lines and fresh human peripheral blood lymphocytes. The mutant drug-resistant virus strains were passaged through C81-66/45 uninfected T cells. Briefly, 4×10^6 T cells were infected with 1 ml of cell-free virus-containing supernatant. The supernatants of virus-producing cells were passed through nitrocellulose filters (pore size, 0.45 μm) aliquoted, and kept frozen at −80°C until use. Infection could be followed by visual inspection, e.g., by formation of syncytia in infected cultures. In addition, viral p24 antigen was measured in the cell culture medium by antigen capture, using a core profile enzyme-linked immunosorbent assay (ELISA) (Du Pont de Nemours, Bad Nauheim, Germany). Supernatants were tested in serial dilutions and p24 values were derived from the linear range. Virus dilution was carried out to determine the infectivity. Transmission was performed with the following MOIs for the different virus strains: HIV-1_{HXB2} parental wild-type strain, MOI 5×10^{-4}; AZT-resistant HIV-1_{RTMN}, MOI 2.5×10^{-3}; 3TC/FTC-resistant HIV-1_{184V}, MOI 5×10^{-4}; and triple drug-resistant HIV-1_{RTMDR1}, MOI 2.5×10^{-4}. All three mutant HIV-1 isolates are descendants of the HIV-1_{HXB2} wild-type strain. Two hours postinfection cells were washed and ODNs were added as described above.

RESULTS

Growth and characterization of HIV-1 isolates

To study the effect of TFO A on HIV isolates with drug-resistant RTs three mutant strains were used. The different viral isolates were first passaged through C81-66/45 T cells. Culture supernatants of the four isolates were used and transmission of the virus was monitored by the increase in p24 viral core antigen in the supernatant of infected cells and by the appearance of syncytia in the infected cultures. The results of these experiments are shown in Table 1. The MOIs used for ensuing experiments were adjusted first according to p24 values measured in the supernatants of infected cultures; a second adjustment depended on the time points of which viral antigen became detectable postinfection (the latter data are not shown). The amount of p24 viral antigen produced is highest in the cell cultures infected with the HIV-1_{HXB2} isolate (6.25×10^4 pg/ml). In the supernatants infected with the drug-resistant mutants generally lower amounts of core antigen are detected. The lowest amount of viral antigen p24 was measured in the cell culture infected with the triple drug-resistant HIV-1 isolates HIV-1_{RTMDR1} (1.1×10^2 pg/ml).

The amount of virus used for this study can be deduced from the titrations of infectivity indicated by the MOI. In general the amount of virus used was one order of magnitude higher than the minimum amount needed to infect the target cells with a particular HIV variant, to ensure that the amount of virus used for the inhibition of HIV replication was always sufficient to establish an infection. In addition, the lower amount of virus used resembles more closely the *in vivo* situation in an HIV-infected individual, in whom the virus concentration in peripheral blood tends to lead to similar low MOIs. Previous studies indicated that the MOI of CD4 lymphocytes *in vivo* probably does not exceed the value of 0.0027.[15,16] The MOI value was calculated from the daily mean turnover rate of the virus (1.1×10^8) and the daily turnover rate of $CD4^+$ lymphocytes (2×10^9), which was calculated to constitute 5% of the total $CD4^+$ lymphocyte population, which would amount to 4×10^{11} cells.[17] These calculations do not take into account other cell populations as possible targets for the virus, such as monocytic cells or endothelial cells. It can therefore be concluded that the real ratio of infectious units to the number of possible cellular targets must be orders of magnitude lower. On the basis of these considerations a low amount of virus was used in these experiments.

Inhibition of HIV-1 drug-resistant isolates by TFOs

To test for antiretroviral activity of triplex-forming ODNs, C81-66/45 cells were inoculated with the HIV-1 mutants at the viral doses mentioned above for 2 hr. Infected cells were then washed and ODNs were added with fresh medium for 24 hr (first spike). Twenty-six hours postinfection the medium was removed and fresh medium was added, again containing the ODNs at the same concentration (2 μM) (second spike). In these experiments the antiviral effect of TFO A was compared with that of a scrambled sequence ODN (TFO SC) of equal composition and chain length (54-mer). The TFO A was modified by phosphorothioate groups at both termini and by the four Ts of the linker. The TFO SC was modified accordingly as previously described.[6] Control cultures were also inoculated with the same viral isolates but incubated without ODNs. Some cultures were kept uninfected for comparison of cytopathic effects.

The antiretroviral activity of TFO A at 2 μM concentration in C81-66/45 cells inoculated with the parental HIV-1_{HXB2} strain is shown in Fig. 1A. No p24 antigen is found in supernatants from this culture during the entire cultivation period. In contrast, p24 viral antigen production is observed in the cells

TABLE 1. CHARACTERIZATION AND GROWTH OF DRUG-RESISTANT HIV ISOLATES

HIV-1 isolate	*Mutation*	*p24 antigen (pg/ml) 10 days postinfection*	*MOI of HIV used for infection*
HIV-1_{HXB2}	—	6.25×10^4	5.0×10^{-4}
HIV-1_{RTMN}	M41L, T215Y	2.21×10^4	2.5×10^{-3}
HIV-1_{184V}	M184V	2.20×10^3	5.0×10^{-4}
HIV-1_{RTMDR1}	M41L, L74V, V106A, T215V	1.10×10^2	2.5×10^{-4}

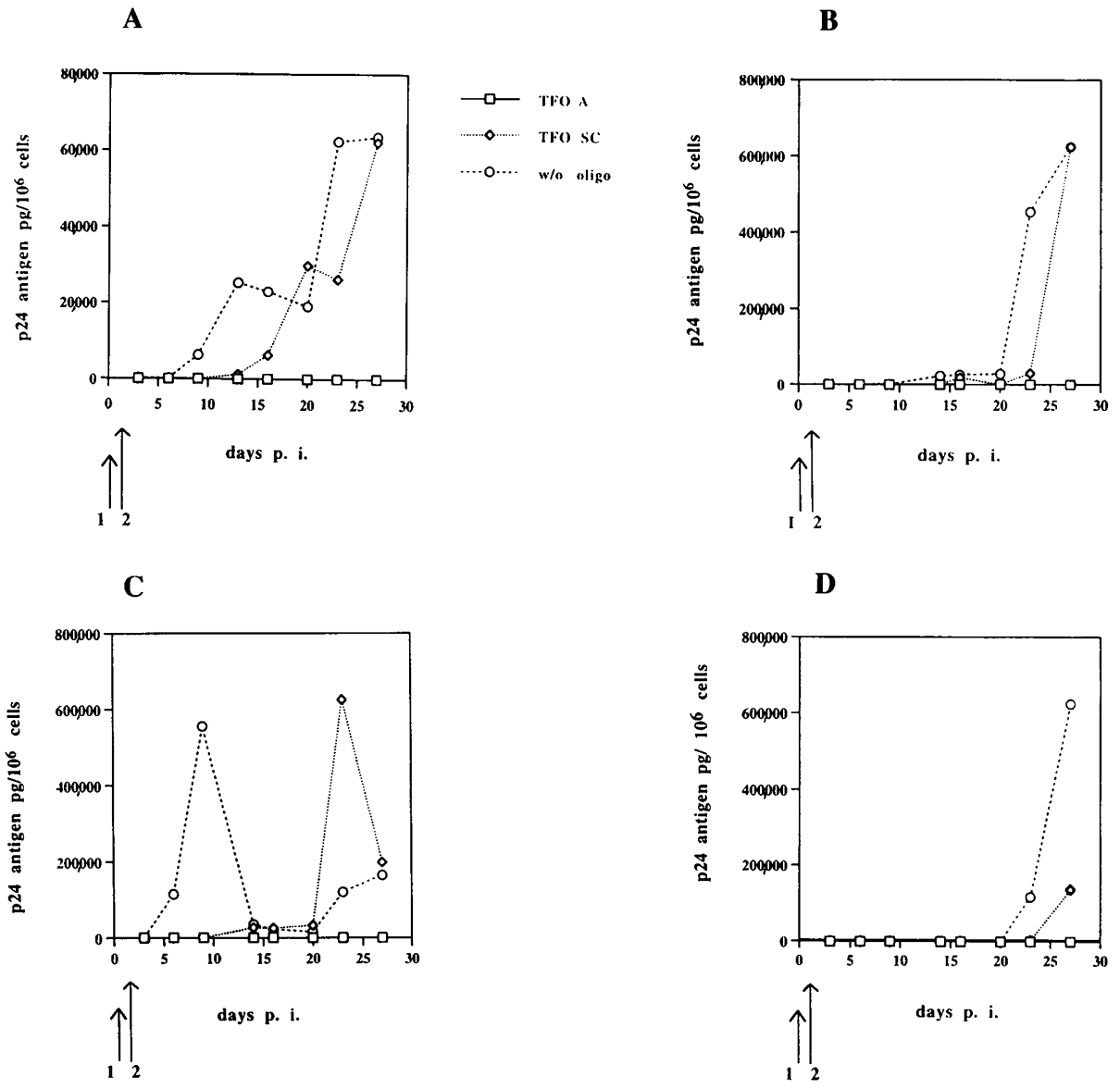

FIG. 1. (A–D) Antiviral effect of phosphorothioate-modified TFO A and a scrambled control TFO SC on HIV-1 replication of three different drug-resistant variants in comparison with the parental strain HIV-1_{HXB2}. Cultures of C81-66/45 T cells were inoculated with the HIV-1_{HXB2} strain for 2 hr. After incubation with the virus, cells were washed and treated with the ODNs (2 μM) (arrow 1). A second treatment with ODNs at identical concentration was performed 24 hr later (arrow 2), and the growth medium was changed. As control, one culture was assayed without added ODN. Supernatants were collected at the times indicated and viral antigen expression was determined by p24 antigen capture assay. The amount of p24 (pg) per 1×10^6 cells was plotted as a function of days postinfection (p.i.). Syncytium formation was monitored by microscope. (A) Antiviral effect of TFO A on HIV-1_{HXB2} wild-type isolate exhibiting drug-sensitive phenotype; (B) antiviral effect of TFO A on AZT-resistant mutant isolate: HIV-1_{RTMN} characterized by the RT mutations M41L and T215Y; (C) antiviral effect of TFO A on 3TC/(–)-FTC-resistant HIV-1_{184V} isolate bearing the RT mutation M184V; (D) antiviral effect of TFO A on triple drug-resistant HIV-1_{RTMDR1} mutant isolate bearing four RT mutations: M41L, L74V, V106A, and T215V. (□) TFO A; (◇) TFO SC; (○) without (w/o) ODN treatment.

treated with the control oligonucleotide TFO SC. Likewise, HIV-1 synthesis is detected in the culture not treated with TFO A. The HIV-1_{HXB2} parental strain exhibits full sensitivity to AZT.

The HIV-1_{RTMN} mutant was tested analogously and exhibited sensitivity to TFO A as shown in Fig. 1B. During the 30-day-long cultivation period of HIV-1_{RTMN}-infected C81-66/45 cells, TFO A treatment led to a total abrogation of virus expression as monitored by p24 antigen synthesis and syncytium formation. In contrast, core antigen expression started in untreated cultures on day 10 postinfection, leading to a sharp rise in virus production by day 20 postinfection. In cultures treated

with the scrambled sequence control TFO SC HIV-1 antigen production is first observed on day 16 postinfection and a massive amount of virus expression is measured 7 days later (23 days postinfection). The effect of TFO A is long-lasting: for 27 days (the entire period of treatment) p24 antigen levels never rose over background values.

The next experiment was carried out to test whether TFO A was also capable of exhibiting an antiviral effect in T cell cultures infected with the HIV-1_{184V} variant; this variant, with mutation M184V, is characterized by resistance against the two nucleoside inhibitors 3TC and (–)-FTC. As can be seen in Fig. 1C, infection of T cells treated with this 3TC/FTC-resistant mutant of HIV-1 leads in untreated cultures to a rapid rise in p24 production starting 3 days postinfection. Supernatants of the culture, however, treated with TFO A at 2 hr post-HIV-1 inoculation, do not contain p24 antigen for up to 27 days. No syncytium formation was detectable. In the control culture receiving an equal amount of TFO SC, HIV p24 synthesis commences 9 days postinfection, and rapid and massive viral protein expression is apparent by day 23 postinfection. It can be seen from the latter two experiments that virus expression in cell cultures treated with TFO SC usually starts 6 or 7 days later, compared with the untreated control cultures. Finally, the triple drug-resistant HIV-1_{RTMDR1} isolate was tested. In addition to AZT resistance this HIV variant isolate was reported to show no sensitivity to inhibition by dideoxyinosine and the nonnucleoside inhibitor nevirapine. T cells were infected with the HIV-1_{RTMDR1} isolate and treated 2 hr later with TFO A. As Fig. 1D shows, no antigen expression can be demonstrated in the supernatant media of infected and TFO A-treated cells. No syncytium formation was detectable. In untreated cell cultures and in cultures that received the control TFO SC, viral core protein expression is observed 20 and 23 days postinfection, respectively, because of the slow replicative capacity of this mutant. Afterwards, a rapid rise in antigen production is observed in both cultures.

DISCUSSION

In HIV-1 genomic RNA the 3′-PPT, a sequence of 16 purine bases, forms a unique tight structure protecting this short stretch of RNA and the opposite DNA from hydrolysis by RNase H.[6] The 3′ end of the PPT RNA can then serve as primer for the second strand (plus-strand DNA synthesis). Addition of TFO A to virus-specific RNA leads *in vitro* to reactions that clearly demonstrate triple-helix formation. TFO A prevents the hydrolytic action of RNase H *in vitro*.[6] In cell culture little is known about the mechanism of action of TFO A on viral replication. We considered the possibility that the inhibitory effect of TFO A on viral replication might be due to G clusters contained within the nucleotide sequence. Contiguous G bases can be responsible for the formation of G tetrades, very stable structures known to interfere with a variety of biological processes.[18,19] However, the scrambled control TFO SC contains six adjacent guanine residues as well, while not exhibiting any antiviral effects. Therefore such a possibility can be excluded. Furthermore, we considered the possibility that TFO A may directly affect enzymatic activities of the RT, especially since TFO A may be able to form a self-complementary hairpin–loop structure.[8] Therefore we chose several mutant viruses with known mutations in the RT to test whether the mutations would influence the effect of TFO A.

We selected three drug-resistant mutants. The mutant HIV-1_{RTMN} is resistant against AZT and exhibits a mutation in codon T215Y. This mutation has been described to affect the function of the RT during polymerization.[11] Cloning and sequencing studies of another strain, HIV-1_{184V}, have identified an M → M mutation at codon 184 that confers 500 to 1000-fold resistance in tissue culture against the enantiomers of 2′,3′-dideoxy-3′-thiacytidine (3TC) and 2′,3′-dideoxy-5′-fluoro-3′-thiacytidine [(–)-FTC]. In addition, this mutation conferred 5- to 10-fold resistance to ddI and ddC.[10] The same researchers were also able to demonstrate the finding of mutations at codon 184 (from M to V or I) after passaging the virus in the presence of 3TC or (–)-FTC. The acquisition of these mutations, which was determined in cell culture, was then also demonstrated in clinical isolates from patients receiving long-term AZT treatment.[20] A third mutant, the triple drug-resistant isolate of HIV-1_{RTMDR1}, has also been included in our investigation. This isolate exhibits resistance against a structurally different nonnucleoside compound, namely nevirapine, that antagonizes HIV RT activity by acting as a noncompetitive inhibitor of DNA chain elongation. Nonnucleoside inhibitors all contain aromatic moieties and bind to the same hydrophobic pocket in HIV RT.[21] Drugs such as nevirapine can inhibit HIV-1 at low concentrations, but 1000-fold resistance has been noted following *in vitro* passage of HIV in the presence of these drugs.[22] In addition, the above-mentioned isolates exhibits resistance against AZT and ddI.

The RT mutations from the three drug-resistant viral isolates all cluster in the amino-terminal half of the enzyme molecule, which is thought to be involved in nucleotide recognition. At present it appears that the mutated residues may be conferring drug resistance by influencing interactions between the protein and the template strand.[23,24] These mutations are located at the polymerase active site and cluster in two regions: M41L, D67N, T69D, K70R, and L74V; and T215Y (or T215F) and K219Q (or K219E). The approximate locations of these two regions, the former on the "fingers" subdomain and the latter on the "palm," suggest that these residues play a role in protein–template interaction. A space-filling model of the RT enzyme has been constructed that indicates that the location of these two regions containing the resistance mutations is placed in close proximity to the template strand of the nucleic acid substrate.[23] Generally, it has been found that nucleoside analog-resistance mutations in the p66 subunit are located in positions that interact with template–primer, incoming dNTP, or nearby residues of the dNTP-binding site. In contrast, mutations in p51 are buried and far away from the DNA-binding cleft, and therefore do not contribute to resistance.[21] All nonnucleoside inhibitor resistance mutations in p66 cluster around the nonnucleoside-binding pocket, while the corresponding mutations in p51 are located far away from the pocket and have no effect on inhibition.[25] Examination of three classes of nonnucleoside RT inhibitors suggested that amino acids 101–106 of the RT interact with residues 155–217.

Structural analyses of the RT and primer–template modeling have been published and demonstrate how the substrate fits into the RT molecule. A grove exists between the RNase H active site and the polymerase active site with its two aspartic acid

residues D185 and D186. DNA synthesis primed with the viral RNA template is accompanied by cleavage of the RNA template, at an estimated 16 to 18 nucleotides downstream of synthesis, by RNase H.[2,26,27] To fit into the groove along its full length, the DNA would have to be bent toward the protein in the middle. In this model there are about 20 base pairs of DNA–RNA hybrid between the primer terminus and the RNase H active site.[23]

We considered the possibility that the TFO A mimicks such a template–primer structure, since the TFO A can theoretically form a hairpin–loop structure that also might fit into the RT model. Furthermore, *in vitro* studies have shown some enzyme inhibition of the RT and the RNase H in primer-extension or hybrid hydrolysis studies, albeit at higher concentrations of TFO A *in vitro*.[6] Lower TFO A concentrations of 1 to 2 μM are effective in culture. The three drug-resistant mutant RTs are all sensitive to the TFO A treatment, as demonstrated here. This suggests that not the RT, but other functions or steps during replication, are involved. Those steps must occur before DNA provirus formation, since we have already shown that this does not happen in the presence of TFO A.[8]

The surprisingly potent effect of TFO A on HIV replication may result from multiple effects on different replicative functions of the virus such as the RT, the RNase H-mediated initiation of the second strand, and/or even the integration step, which is mediated by the integrase. This latter possibility is considered because the 3′-PPT is located adjacent to the recognition site of the integrase. Further mutational analyses may reveal to what extent a real triple-helix effect on the PPT is involved.

The PPT has also been selected by other researchers as a target for triple-helix formation. Those authors used a TFO conjugated with psoralen and introduced into the nuclei of permeabilized cells with subsequent ultraviolet irradiation, which resulted in nuclease resistance; this suggests binding of the TFO to the PPT.[28] An earlier attempt to repress transcription by triplex formation was performed by Cooney *et al.*[29] by using an ODN directed against a purine-rich upstream region in the c-*myc* promoter. Thus information on the intracellular efficiency of triple-helix function is limited. We are presently analyzing a number of point mutations and compensating mutations in TFO A to learn more about the molecular basis of this surprisingly potent inhibitor of HIV replication.

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