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Silencing of HIV by hairpin-loop-structured DNA oligonucleotide

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Abstract We describe inhibition of HIV replication by a partially double-stranded 54mer oligodeoxynucleotide, ODN, which consists of an antisense strand targeting the highly conserved polypurine tract, PPT, of HIV, and a second strand, compatible with triple-helix formation. Upon treatment of HIV-infected cells with ODN early after infection no viral nucleic acids, syncytia or p24 viral antigen expression was observed. The ODN-mediated effect was highly sequence-specific. The ODN against HIV-IIIB was effective preferentially against its homologous PPT and less against the PPT of HIV-BaL differing in two of 24 nucleotides and vice versa. It may be interesting mechanistically as an antiviral drug.

Keywords: Polypurine tract; Triple-helix; Sequence-specific RNA degradation; Reverse transcriptase-RNase H; siRNA

1. Introduction

Retroviruses replicate their viral RNA by concerted action of the reverse transcriptase (RT) and ribonuclease H (RNase H). While the RT is synthesizing the complementary cDNA the RNase H specifically hydrolyzes the viral RNA in RNA–DNA hybrids [1–3]. Hereby a special region, the polypurine tract, PPT, resists to hydrolysis and remains hybridized to the cDNA [4–6]. The PPT-RNA is used as RNA primer for initiation of the second strand DNA synthesis and removed later. The PPT is located adjacent to the unique region at the 3′-end (U3). It is one of the most highly conserved sequences of HIV. The PPT is 25 nucleotides long in its extended form and consists of two polypurine clusters interrupted by two non-purines (CU), which are located adjacent to an internal A. 5′ of this ACU triplet is the cleavage site of the viral RNase H (Fig. 1A) [4], the start site for the RT for the second strand DNA synthesis. Furthermore, the PPT sequence is important at later stages during replication for recognition by the integrase and integration of the DNA provirus [7]. Thus, ACU is a sequence of extreme functional importance for three viral enzymes, RT, RNase H, and integrase. The PPT RNA–DNA heteroduplex has some structural peculiarities [8,9] and may therefore be a preferred recognition site of the RT/RNase H.

We considered the PPT a good target for intervention of HIV replication and designed an oligodeoxynucleotide (ODN) targeted to the PPT-RNA consisting of an antisense strand of 25 nucleotides, linked by four thymidines to a second strand, which was designed on the basis of Hoogsteen rules for purines [10] with the exception of the two non-purines CU. This ODN is a partially self-complementary hairpin-loop-structured DNA, 54 bases in length (Fig. 1), containing two small loops, a linker and non-hybridizing ends [6,11,12]. We showed previously that this ODN, designated as ODN A, inhibited HIV production, which we interpreted as an antiviral triple-helix effect [11,12]. The efficiency of the antiviral effect was surprising, long-lasting, superior to an antisense effect, and without viral breakthrough for several weeks. Furthermore, patient isolates in peripheral blood mononuclear cells (PBMCs) or viral isolates with drug-resistant RT mutants were sensitive to this antiviral effect. No DNA provirus was detectable [11,12]. Furthermore, we described previously in vitro studies on the effect of ODN A on the PPT-RNA. At low concentrations of ODN A cleavage occurred, while high concentrations were inhibitory [6]. We anticipate the intracellular levels of ODN A to be very low.

Here we extend our previous studies on the antiviral effect of a hairpin-loop-structured DNA by analyzing a series of mutants of the ODN A. We demonstrate that the sequence and length of each of the two strands is important, excluding a simple antisense mechanism. We are describing a phenomenon, which leads to silencing of viral nucleic acid early after infection and prevents DNA provirus formation. It is reminescent of siRNA and may be designated as "siDNA".

2. Materials and methods

2.1. Cells and viruses

The cellular assays were performed with the HTLV-1-transformed T-cell line C81-66/45 [13], kindly provided by R.C. Gallo (Institute of Human Virology, Baltimore, MD, USA) and PM1, a $CD4^+$T-cell clone derived from the neoplastic cell line HUT 78, kindly provided by P. Lusso (San Raffaele Scientific Institute, Milan, Italy) [14]. Cells were propagated as described [12]. The HIV-1 viral strains used were HIV-1 IIIB and the macrophage-monocyte-tropic viral isolate HIV-1 BaL.

2.2. Oligodeoxynucleotides

The ODNs (see Fig. 1) are targeted to the extended polypurine tract, PPT, of HIV-1 located at the 3′ end of the genome, not the central one [15], which is slightly different. They consist of a

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Abbreviations: as, antisense; ODN, oligodeoxyribonucleotide; PPT, polypurine tract; RNase H, ribonuclease H; RT, reverse transcriptase; siRNA, small interfering RNA

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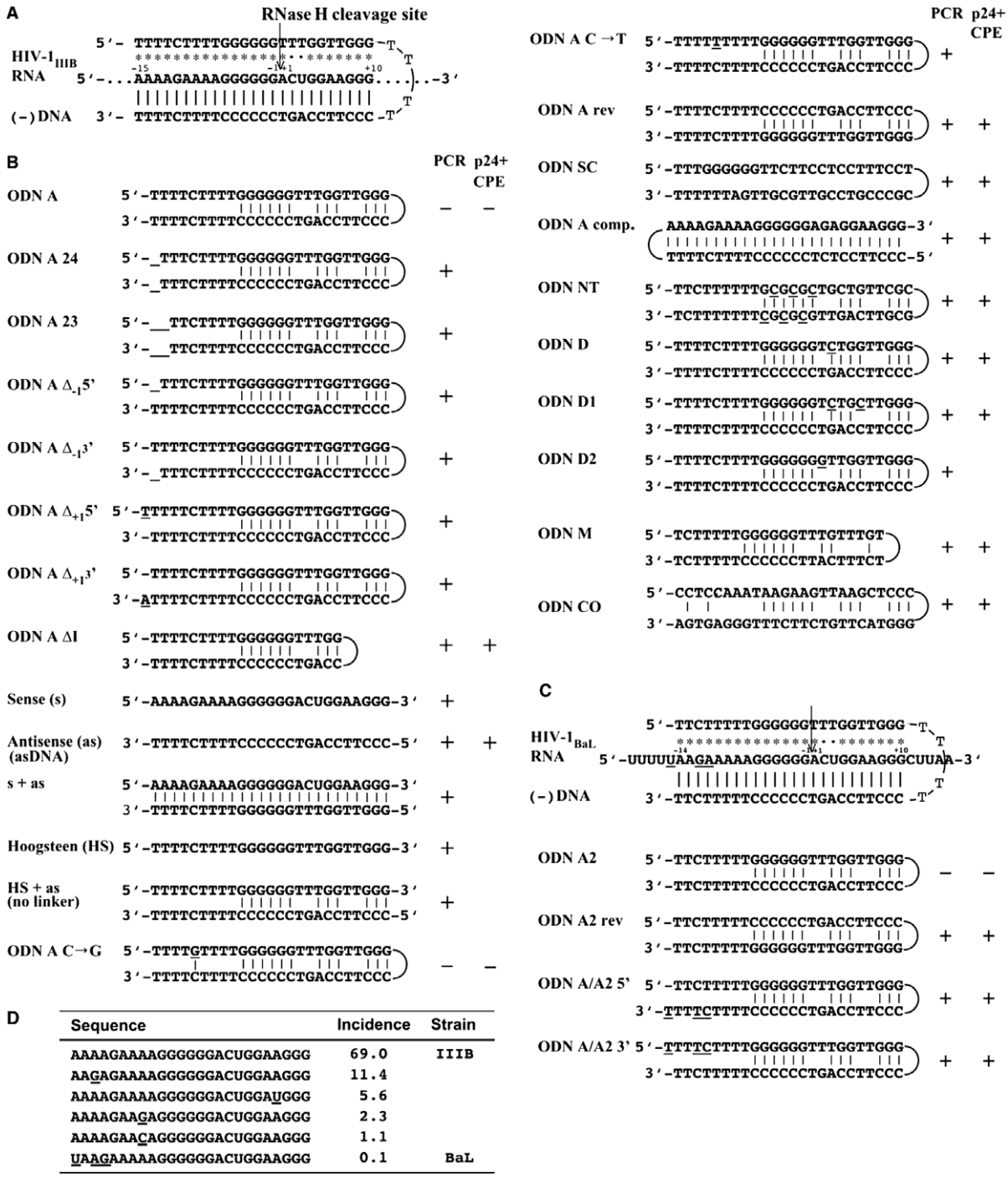

Fig. 1. Sequences of ODNs. (A) The sequence of the extended polypurine tract, PPT, is shown with the cleavage site of the RNase H, 5′ of the ACU sequence (vertical arrow, origin of coordinates). The ODN A consists of an antisense "Watson–Crick" strand and a "Hoogsteen" strand linked by four thymidines. A putative triple-helix is shown. Watson–Crick bonds are shown by vertical bars, Hoogsteen bonds by stars, two non-Hoogsteen bonds by dots. (B) The sequences of ODN A and its variants are depicted in hypothetical partially self-complementary hairpin-loop structures. Curved lines symbolize the linker consisting of four thioated thymidines. Cells were infected with HIV IIIB and two hours later treated with ODNs (2 μM). Supernatants were collected for two to three weeks and viral core antigen p24 determined by ELISA, cytopathic effect (CPE) by light microscopy. The PCR results listed are summarized from Figs. 2 and 3 or data not shown. (+) indicates presence of PCR product, CPE and viral growth, and (−) negative results due to antiviral effect. *Abbreviations*: I, internal deletion; rev, reverted; sc, scrambled; comp, complementary; NT, no tetrades; underlines indicate changes. ODN M and ODN T were used as control. (C) Same as Fig. 1A and B except for HIV-BaL. (D) Sequence alignment of PPTs. Numbers indicate the incidence of the sequences in 2094 PPT of HIV-1 subtype B analyzed.

25mer antisense, and a 25mer putative triple helix-forming 'Hoogsteen' strand, connected by four thymidines. The sequence is partially complementary (see Fig. 1B). The ODNs were phosphorothioated at either end (3 bases each), and in the T4 linker. The ODNs were purchased from MWG-Biotech, Ebersberg, Germany.

2.3. Inhibition of viral expression in acutely HIV-infected cells by ODN A in cell culture

Experiments were performed as described previously [11,12]. Briefly, 2×10^5 cells of the T-lymphocyte cell-line C81-66/45 were infected with HIV-1 IIIB strain [13], with a MOI of 0.01. In a similar way 2×10^5 PM1 cells were infected with HIV-1 BaL [14] with a MOI of 0.1. One to two hours post infection cells were washed and treated with ODNs at 2 μM concentration or 1 μM for ODN A2, respectively. In the case of Fig. 3B ODNs were added twice, 2 and 24 h post infection. Cells were kept as described [11,12]. Syncytia formation was monitored by microscopy and viral p24 antigen determined as described previously [12].

2.4. Determination of HIV viral nucleic acids by nested PCR, RT-PCR and real time PCR

Kinetic analysis of RNA/DNA intermediates of replication was carried out with C81-66/45 cells, which were infected with HIV-1 IIIB, or PM1 cells infected with HIV-1 BaL as described above, and incubated as indicated. ODNs were added to a final concentration of 2 μM at 0.5 or 1 h after infection. Samples were taken before infection or post infection at the times indicated. For isolation of nucleic acids RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and for RT-PCR or PCR Access RT-PCR Kit (Promega, Madison WI, USA) was used according to the manufacturer. Extracted nucleic acids consisted of RNA and DNA. Kinetics of virus infection for HIV-1 IIIB were analyzed using the primer sets PU3, which gives rise to PCR products of 136 bp (nested), PPU primers giving rise to 612 bp and 109 bp (nested), and PPT-B primers leading to 273 bp (see schemes in Fig. 2A–C). Furthermore, the primers PU3.1 and PU3.4 yields a product of 328 bp (Fig. 2D, top). Primers for PU3 were: outer primers: PU3.1 (23–42 bp): 5′-GTAGTGTGATTGGATGGCCT and PU3.2 (891–910 bp): 5′-CCACACTGACTAAAAGGGTC, inner primers: PU3.3 (214–243 bp): 5′-CCTCAGGTACCTTTAAGACC and PU3.4 (331–350 bp): 5′-GTGTGGTAGATCCACAGATC; for PPU: outer primers: PPU-1 (298–320 bp): 5′-CTAATTCACTCCCAAAGAAGACA and PU3-2 (see above), inner primers: PPU-2 (331–350 bp): 5′-GATCTGTGGATCTACCACAC and PPU-3 (421–440 bp): 5′-GGTACTAGCTTGTAGCACCA; for PPT-B: outer primers: PU3-1 (see above) and PPT-B (277–296 bp): 5′-CCTTCCAGTCCCCCCTTTTC. For HIV-BaL the primers used were BaL-1 (3217–3236 bp) 5′-GATGGGTGGCAAGTGGTCAA and BaL-2 (3611–3630 bp) 5′-GTCAGTGGAAATCTGGTCCC, giving rise to 413 bp. Coordinates for HIV-1 IIIB-specific primers refer to Accession No. X03187 and for BaL-specific primers to Accession No. M68893. A control for equal recovery of RNA was performed using GAPDH primers: GAPD-1: 5′-CCGTCTAGAAAAACCTGCCA and GAPD-2: 5′-GAGCTTGACAAAGTGGTCGT. Primers were purchased from Birsner and Grob – Biotech GmbH, Freiburg, Germany. Controls (Cont. in Figs. 2, 3) were performed without ODN and virus (0 h) or with virus indicated by time post infection (1 or 0.5 h).

The amount of HIV-specific RNA was quantified by a real-time PCR assay (Fig. 2D, bottom) using the ABI 7300 instrument (Applied Biosystems, Foster City, CA, USA). Sequences of PCR primers (which cover region close to PPT) and probe were the following: For1 5′-GAGGAGGTGGGTTTTCCAGT, Rev1: 5′-GGGAGTGAATTAGCCCTTCC, probe: FAM-ACCTTTAAGAACCAATGACTTACAAGGCAGC-TAMRA. Primers were purchased from Operon, Cologne, Germany. The RT-PCR reactions were performed with the AMV-RT from a commercially available kit (Promega Co). The test system was not affected by ODNs (data not shown). The sequence of the control ODN CO targeted to a region 10 nucleotides downstream

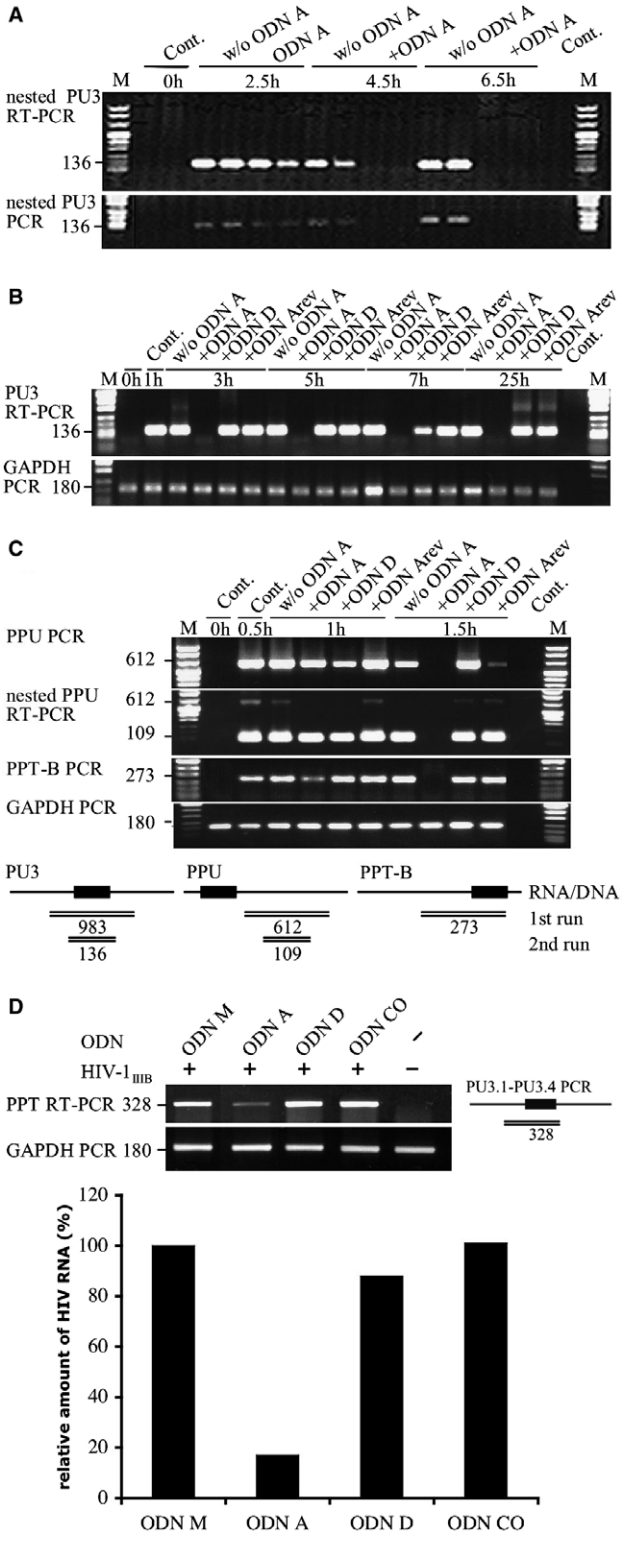

Fig. 2. Analysis of viral nucleic acids during infection. (A) Cells infected with HIV-1 IIIB were treated with ODN A for the indicated periods of time post infection. Nested RT-PCR products (upper panel, 136 bp) or PCR products (lower panel, 136 bp) using primer set PU3 are shown in duplicates. The reactions for different primer sets are depicted schematically at the bottom. (B) Longer term analysis of cells infected with HIV-1 IIIB treated 2 h post infection with the indicated ODNs and analyzed 3, 5, 7, and 25 h later. RT-PCR of GAPDH is shown as control. (C) Short term analysis of HIV-1 IIIB-infected cells treated with ODN by PCR, nested PCR or RT-PCR using primer sets PU3 flanking the PPT, PPU for downstream (612 and 109 bp) and PPT-B for upstream regions of the PPT (273 bp) and GAPDH (180 bp) as control. (D top) An RT-PCR reaction using primers PU3.1 and PU3.4 leading to a 328 bp PCR fragment as shown in the scheme was performed by using HIV-1-infected cells 7 days post infection and two treatments of ODNs (time 0 and 2 h post infection). The lower panel shows GAPDH PCR control reactions. (D bottom) The graph shows the analysis by real time PCR for quantification of the RNA using primer sets as described in Section 2.

of the PPT, is: 3′-AGTGAGGGTTTCTTCTGTTCATGGG(T_4)-CCCTCGAATTGAAGAATAAACCTCC-5′. It has a putative hairpin-loop structure similar to ODN A (see Fig. 1).

3. Results

3.1. Inhibition of HIV-1 replication by ODN A

HIV-1 replication was analyzed in the T-cell line C81-66/45 T-cells [13] by infecting them with HIV-1 IIIB. Subsequently the cells were treated with ODNs. The sequential order should prevent their interference with viral entry. A number of ODN mutants were designed and are shown as hypothetical partial self-complementary structures containing two bulges, non-hybridizing ends and a T4 linker. The ODN A consists of an antisense strand and a second strand with a selection of nucleotides based on Hoogsteen bonds. Several ODNs are presented and nucleotide changes are indicated by underlining (Fig. 1A and B). The effects of several ODNs were determined by PCR, ELISA for p24 core antigen, cytopathic effect (CPE), or syncytia formation as marker for HIV (listed in Fig. 1B). Two controls not related to the PPT are shown, an ODN M (against a murine viral PPT) and an ODN CO, targeted to a region 10 bases downstream of the PPT. ODNs specific for HIV-BaL and controls are shown in Fig. 1C. The HIV PPTs were aligned using the Los Alamos database, showing their importance by their high degree of conservation (Fig. 1D).

Among the ODNs listed only ODN A and one variant, ODN A(C to G), were able to inhibit p24 antigen production, syncytia formation, and PCR or RT-PCR product synthesis. The C to G exchange would be compatible with triple-helix rules. However, ODN D or the shortened ODNs are as compatible with a triple-helix design as ODN A – yet are ineffective. Therefore we do not know whether a triple-helix is involved. All other changes of ODN A abrogated the inhibitory effect, such as shortening of the ends, exchanges of nucleotides, or changes in number of bonds (ODN D and D1). A nucleotide exchange near the 5′end (ODN A(C to T)), or internally (ODN D and D2), abrogated the antiviral effects. These ODNs have antisense strands like ODN A and are fully complementary to the RNA. Yet their second arms are mutated, which abrogates their antiviral efficacy. Surprising was the importance of the sequence and length of the second arm, demonstrating that the mechanism is not only a classical antisense effect. It is reminescent of siRNA.

In order to analyze the time course of the effect of the ODNs we infected cells with HIV-IIIB at time 0, and treated them with ODNs without carrier 0.5 h later, to prevent interference of ODNs with virus uptake. The cells were lysed 2.5, 4.5, 6.5 h after infection and PCR or RT-PCR analysis were performed (Fig. 2A). We also tested longer periods for up to 25 h (Fig. 2B) or shorter periods (Fig. 2C). The time course shows some variation at early time points depending on the individual experiments. As internal standards we used GAPDH as controls. The primers were selected to allow amplification products spanning the PPT, another pair downstream of the PPT, and a third one including the PPT. The reactions are indicated schematically at the bottom. The result shows that the presence of ODN A resulted in disappearance of HIV-specific nucleic acids very early after virus infection.

A comparison between RT-PCR and quantitative real time PCR was performed with C81-66/45 cells infected with HIV and treated with the ODNs at time 0 and 2 h post infection. The cells were lysed after 7 days for the assay. The result of

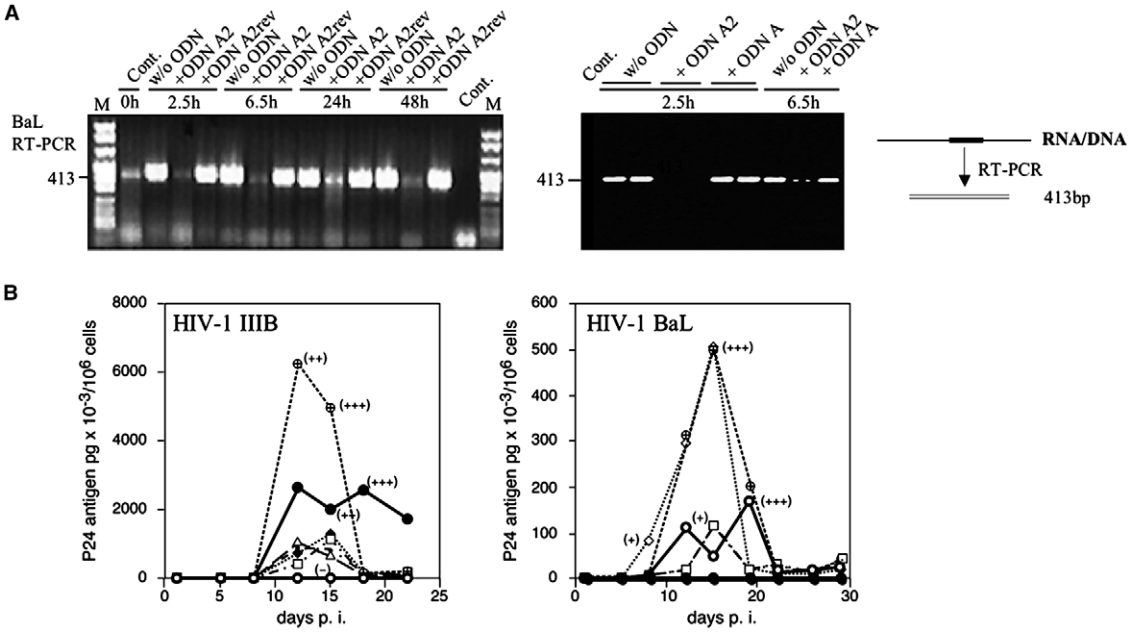

Fig. 3. Analysis of ODNs on HIV-1 BaL infected cells. (A) Similar to Fig. 2B except that HIV-1 BaL and PM1 cells were used and RT-PCR was performed using the primer set BaL yielding a 413 bp product after the 1st run. The HIV-1 BaL specific ODN A2 was tested in comparison to the control ODN A2rev (left) and compared with the HIV-1 IIIB-specific ODN A (right). (B) p24 antigen determination as marker for HIV in the supernatant from C81/66-45 cells infected with HIV-IIIB (left) and PM1 cells infected with HIV-1 BaL (right). Virus was added at time 0, ODNs were added twice, 2 and 24 h later. Left: ODN A (○), ODN A2 (●), ODN A2rev (◇), ODN D (△), ODN sc (□), no ODN (⊕), right: same as left except ODN A2 (2 μM and 1 μM, black and grey dots) and no ODN D. Syncytia formation was evaluated by microscopic inspection and was graded (+++), (+), (+/−), or (−) with decreasing numbers [7,8].

the analysis shows a strongly reduced virus-specific signal with ODN A and not with the control ODNs, as determined by RT-PCR analysis (Fig. 2D, top) and quantitative real-time PCR (Fig. 2D bottom). The primer sets are given in Section 2.

3.2. Homologous inhibition

To further characterize the ODN A-specific antiviral effect, we investigated a naturally occurring virus variant. We screened the Los Alamos database for naturally occurring variants of the PPT. 69% out of 2094 variants of HIV-1 were 100% identical in their extended PPT sequence. We selected a natural variant, HIV-1 BaL [14], which contains a PPT consisting of 24 instead of 25 nucleotides and two exchanges at position −11 and −12, (GA instead of AG) compared to HIV-1 IIIB (Fig. 1C).

We designed an ODN A2 against the corresponding extended PPT of HIV-1 BaL (Fig. 1C), which was tested on the homologous strain HIV-1 BaL using PM1 cells. The ODN A2 was added 0.5 h post infection, the cells were lysed at the times indicated and RT-PCR analysis was performed (Fig. 3A). As can be seen no PCR product is detectable with the ODN A2 in contrast to a control ODN A2rev (left) and ODN A (right).

We then tested ODN A2 as well as ODN A on both virus strains in long-term cultures by infecting C81-66/45 cells with HIV-1 IIIB and PM1 cells with HIV-1 BaL. The viruses were added at time 0, the ODNs were added twice, 2 and 24 h later as described previously [12]. Inhibition of viral replication was observed much more efficiently for the homologous than for the heterologous combinations of virus and ODNs as determined by p24 antigen expression and syncytia formation (Fig. 3B). We conclude, that the antiviral effect of the ODNs on virus replication is highly sequence-specific.

4. Discussion

We describe the disappearance of viral nucleic acids shortly after HIV infection due to treatment with a highly specialized DNA oligonucleotide, ODN A. The design of the ODN A was originally based on rules for a triple-helix [10], and led to a hypothetical partially self-complementary structure with two bulges and unpaired ends in the absence of a target RNA. We do not know whether a triple-helix is formed inside the cell, because the two non-purines and DNA–RNA–DNA hybrids may not be stable or may only form transiently. Triple-helix formation inside a cell has never been shown to occur [16,17]. A triple-helix has been described previously in vivo for a subregion of the PPT DNA of HIV, however, only after cross-linking and stabilization of the structure before insertion into the cell [16]. Based on our previous in vivo observations [11,12] and a later report [18], we performed studies with a number of mutants of the ODN and on the time course of the effect. Even in cases where the antisense strand was identical to that of ODN A and fully complementary to the viral RNA no antiviral effect was observed, suggesting some mechanism not only based on a classical antisense effect, since the second arm is also important.

We attribute the ODN A-mediated antiviral effect to inhibition of DNA provirus formation [12]. The molecular mechanism behind it may be complex. Preliminary evidence indicates that the viral RT/RNase H contributes to the antiviral effect. The enzyme is specialized to recognize a hybrid at the PPT for initiation of the second DNA strand. Such a hybrid may be formed by ODN A binding to the viral RNA, thereby allowing cleavage of the RNA by the viral RNase H. Primer extension may then lead to further hydrolysis of the viral RNA. This would destroy the viral RNA before a correct cDNA copy was made. The observed importance of the length of the ODN A may be due to structural constraints of the RT/RNase H to fit to the PPT [4,8].

Also the cellular RNase H1 may contribute to such a hydrolysis if a transient RNA–DNA hybrid is formed [19]. The importance of the second arm of the ODN and its sequence-specificity may be attributed to protection against nucleases until it is targeted to the viral RNA.

We even consider the possibility that the RNase H-like activity assigned recently to the PIWI domain of the Argonaute protein, may contribute to the antiviral effect. The PIWI domain is the active component for cleavage of the target RNA by small interfering RNA. It has an RNase H-like structure and an RNase H activity under special assay conditions [20,21]. Unspecific interference of the ODNs with viral uptake can be excluded because inhibition of each virus strain occurred preferentially by its homologous ODNs.

An RNA–DNA hybrid formation is thermodynamically favored over double-stranded DNA, especially since we used a hairpin-loop-structured DNA [22], which may more easily open up than a double-strand. Hybridization may even be further supported by the presence of the nucleocapsid. We and others have shown that the nucleocapsid is a matchmaker or chaperone, which strongly enhances the rate of cleavage and turnover of viral RNA by a ribozyme [23,24]. It may play a role here as well. Whether the nucleocapsid could also support a triple-helix formation is not known. We cannot easily test such a structure to evaluate its contribution to the antiviral effect.

In summary, we describe an antiviral effect by a highly specialized hairpin-loop-structured ODN. The partial double-strand may protect the ODNA against nucleases. When targeted to the PPT several enzymes of viral and cellular origin may contribute to the antiviral effect. Since the PPT is one of the most highly conserved regions of HIV, the ODNA may be interesting for further studies or potentially for development of a drug.

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References

[1] Moelling, K., Bolognesi, D.P., Bauer, H., Büser, W., Plassman, H.W. and Hausen, P. (1971) Association of the viral reverse transcriptase with an enzyme degrading the RNA moiety of RNA–DNA hybrids. Nature New Biol. 234, 240–243.
[2] Hansen, J., Schulze, T., Mellert, W. and Moelling, K. (1988) Identification and characterization of HIV-specific RNase H by monoclonal antibody. EMBO J. 7, 239–243.
[3] Tisdale, M., Schulze, T., Larder, B.A. and Moelling, K. (1991) Mutations within the RNase H domain of human

immunodeficiency virus type 1 reverse transcriptase abolish virus infectivity. J. Gen. Virol. 72, 59–66.
[4] Wohrl, B.M. and Moelling, K. (1990) Interaction of HIV-1 ribonuclease H with polypurine tract containing RNA–DNA hybrids. Biochemistry 29, 10141–10147.
[5] Wohrl, B.M., Volkmann, S. and Moelling, K. (1991) Mutations of a conserved residue within HIV-1 ribonuclease H affect its exo- and endonuclease activities. J. Mol. Biol. 220, 801–818.
[6] Volkmann, S., Jendis, J., Frauendorf, A. and Moelling, K. (1995) Inhibition of HIV-1 reverse transcription by triple-helix forming oligonucleotides with viral RNA. Nucleic Acids Res. 23, 1204–1212, (Erratum in: Nucleic Acids Res. 23, 3804).
[7] Reicin, A.S., Kalpana, G., Paik, S., Marmon, S. and Goff, S. (1995) Sequences in the human immunodeficiency virus type 1 U3 region required for in vivo and in vitro integration. J. Virol. 69, 5904–5907.
[8] Sarafianos, S.G., Das, K., Tantillo, C., Clark Jr., A.D., Ding, J., Whitcomb, J.M., Boyer, P.L., Hughes, S.H. and Arnold, E. (1996) Crystal structure of HIV-1 reverse transcriptase in complex with a polypurine tract RNA:DNA. EMBO J. 20, 1449–1461.
[9] Xiong, Y. and Sundaralingam, M. (1998) Crystal structure and conformation of a DNA–RNA hybrid duplex with a polypurine RNA strand: d(TTCTTBr5CTTC)-r(GAAGAAGAA). Structure 6, 1493–1501.
[10] Malvy, C., Harel-Bellan, A. and Pritchard, L.L., Eds., (1999). Triple Helix Forming Oligonucleotides, Kluwer Academic Publishers, Boston/Dordrecht/London.
[11] Jendis, J., Strack, B. and Moelling, K. (1998) Inhibition of replication of drug-resistant HIV type 1 isolates by polypurine tract-specific oligodeoxy-nucleotide TFO A. AIDS Res. Hum. Retroviruses 14, 999–1005.
[12] Jendis, J., Strack, B., Volkmann, S., Boni, J. and Moelling, K. (1996) Inhibition of replication of fresh HIV type 1 patient isolates by a polypurine tract-specific self-complementary oligodeoxynucleotide. AIDS Res. Hum. Retroviruses 12, 1161–1168.
[13] Salahuddin, S.Z., Markham, P.D., Wong-Staal, F., Franchini, G., Kalyanaraman, V.S. and Gallo, R.C. (1983) Restricted expression of human T-cell leukemia-lymphoma virus (HTLV) in transformed human umbilical cord blood lymphocytes. Virology 129, 51–64.
[14] Lusso, P., Cocchi, F., Balotta, C., Markham, P.D., Louie, A., Farci, P., Pal, R., Gallo, R.C. and Reitz Jr., M.S. (1995) Growth of macrophage-tropic and primary human immunodeficiency virus type 1 (HIV-1) isolates in a unique CD4+ T-cell clone (PM1): failure to downregulate CD4 and to interfere with cell-line-tropic HIV-1. J. Virol. 69, 3712–3720.
[15] Charneau, P., Alizon, M. and Clavel, F. (1992) A second origin of DNA plus-strand synthesis is required for optimal human immunodeficiency virus replication. J. Virol. 66, 2814–2820.
[16] Faria, M., Wood, C.D., Perrouault, L., Nelson, J.S., Winter, A., White, M.R., Helene, C. and Giovannangeli, C. (2000) Targeted inhibition of transcription elongation in cells mediated by triplex-forming oligonucleotides. Proc. Natl. Acad. Sci. 97, 3862–3867.
[17] Vasquez, K.M., Narayanan, L. and Glazer, P.M. (2000) Specific mutations induced by triplex-forming oligonucleotides in mice. Science 290, 530–533.
[18] Hiratou, T., Tsukahara, S., Miyano-Kurosaki, N., Takai, K., Yamamoto, N. and Takaku, H. (1999) Inhibition of HIV-1 replication by a two-strand system (FTFOs) targeted to the polypurine tract. FEBS Lett. 456, 186–190.
[19] Gaidamakov, S.A., Gorshkova, I.I., Schuck, P., Steinbach, P.J., Yamada, H., Crouch, R.J. and Cerritelli, S.M. (2005) Eukaryotic RNases H1 act processively by interactions through the duplex RNA-binding domain. Nucleic Acids Res. 33, 2166–2175.
[20] Song, J.J., Smith, S.K., Hannon, G.J. and Joshua-Tor, L. (2004) Crystal structure of Argonaute and its implications for RISC slicer activity. Science 305, 1434–1437.
[21] Yuan, Y.-R., Pei, Y., Ma, J.-B., Kuryavyi, V., Zhadina, M., Meister, G., Chen, H.-Y., Dauter, Z., Tuschl, T. and Patel, D.J. (2005) Crystal structure of *A. aeolicus* Argonaute, a site-specific DNA-guided endoribonuclease, provides insights into RISC-mediated mRNA cleavage. Mol. Cell 19, 405–419.
[22] Tsuchihashi, Z. and Brown, P.O. (1994) DNA strand exchange and selective DNA annealing promoted by the human immunodeficiency virus type 1 nucleocapsid protein. J. Virol. 68, 5863–5870.
[23] Herschlag, D., Khosla, M., Tsuchihashi, Z. and Karpel, R.L. (1994) An RNA chaperone activity of non-specific RNA binding proteins in hammerhead ribozyme catalysis. EMBO J. 15, 2913–2924, (Erratum in: EMBO J. 13, 3926).
[24] Müller, G., Strack, B., Dannull, J., Sproat, B., Surovoy, A., Jung, G. and Moelling, K. (1994) Amino acid requirements of the nucleocapsid protein of HIV-1 for increasing catalytic activity of a Ki-ras ribozyme in vitro. J. Mol. Biol. 242, 422–429.