Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7706–7710, November 1987
Medical Sciences

Phosphorothioate analogs of oligodeoxynucleotides: Inhibitors of replication and cytopathic effects of human immunodeficiency virus

MAKOTO MATSUKURA*, KAZUO SHINOZUKA†, GERALD ZON‡§, HIROAKI MITSUYA*, MARVIN REITZ¶, JACK S. COHEN†, AND SAMUEL BRODER*

*Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; †Clinical Pharmacology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; ‡Molecular Pharmacology Laboratory, Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD 20892; and ¶Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by Maurice R. Hilleman, July 13, 1987

ABSTRACT **Nuclease-resistant phosphorothioate analogs of certain oligodeoxynucleotides have been tested *in vitro* as antiviral agents against human immunodeficiency virus (HIV) in human T cells. Phosphorothioate analogs complementary to HIV sequences, as well as noncomplementary analogs including homooligomers, exhibited potent antiviral activity. The antiviral activity was related to the base composition of the analogs, and longer phosphorothioates were more effective than shorter ones. A 28-mer phosphorothioate oligodeoxycytidine (*S*-dC$_{28}$) at a concentration of 1 μM exhibited potent antiviral activity and inhibited *de novo* viral DNA synthesis as shown by Southern blot analysis. However, *S*-dC$_{28}$ failed to inhibit *gag* expression in chronically infected T cells assessed by immunofluorescent assay at concentrations up to 25 μM. An N^3-methylthymidine-containing phosphorothioate analog, which does not hybridize efficiently *in vitro* to complementary normal DNA, showed no antiviral activity. A 14-mer phosphorothioate oligodeoxycytidine (*S*-dC$_{14}$) synergistically enhanced the antiviral activity of 2′,3′-dideoxyadenosine, an anti-HIV nucleoside. Therefore, phosphorothioate analogs of oligodeoxynucleotides could represent a unique class of experimental therapeutic agents against the acquired immunodeficiency syndrome and related diseases. However, their mechanism of action is likely to be complex.**

Oligodeoxynucleotides (ODNs; Fig. 1 *Left*), which are complementary to certain gene messages or viral sequences, referred to as "antisense" compounds, have been reported to have inhibitory effects against Rous sarcoma virus (1, 2) and HTLV-III (3), now called HIV. However, the susceptibility of the phosphodiester linkage in n-ODNs (Fig. 1 *Left*, I) to degradation by nucleases (4) would be expected to reduce their potency and *in vivo* persistence as antiviral agents. *M*-ODNs analogs (Fig. 1 *Left*, II) are resistant to nucleases and, being uncharged, have increased hydrophobicity, which reportedly confers increased cell membrane permeability upon these compounds (5, 6). *M*-ODNs have been found to exhibit antiviral activity but may require high concentrations (typically 100–300 μM) to elicit strong antiviral effects (5). *S*-ODNs, in which one of the nonbridging oxygen atoms in each internucleotide phosphate linkage is replaced by a sulfur atom (Fig. 1 *Left*, III), have several properties that make them potentially advantageous antiretroviral analogs. They are stable to cleavage by nucleases (7) and, having the same number of charges as n-ODNs, have good aqueous solubility. They also exhibit more efficient hybridization with a complementary DNA sequence than the corresponding methylphosphonate analogs (unpublished data). These factors led us to test certain *S*-ODNs as antiviral agents against HIV.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Synthesis and Purification of ODNs and Their Methylphosphonate and Phosphorothioate Analogs. The n-ODNs, *M*-ODNs, and *S*-ODNs were synthesized by either the standard procedure or by modification of the reported procedure (8) using an automated synthesizer (Applied Biosystems, Foster City, CA; model 380-B) by the phosphoroamidite method. Purification was performed by reverse-phase high-performance liquid chromatography. The details of synthesis and purification of *M*-ODNs and *S*-ODNs will be described elsewhere. The presence of P—S bonds in the phosphorothioates was shown using ^{31}P NMR spectroscopy (8). N^3-methylthymidine was prepared according to a published procedure (9), then converted to the protected phosphoroamidite form, and incorporated into the oligomer synthesis.

Sequences of ODNs Tested. The sequences of ODNs tested (Fig. 1 *Right*) were chosen for the following reasons; ODN-1 and -3 are complementary (antisense) sequences to regions of *art/trs* genes of HIV (HTLV-III BH10 clone) that are essential for viral replication (10–13). ODN-2 is the "sense" counterpart of ODN-1. ODN-4 is a "random" sequence that has the same base composition as ODN-1, but has <70% homology with any sequence in HTLV-III BH10 clone either as a sense or antisense sequence. The factors of base composition and chain length were evaluated with homooligomers of deoxyadenosine, deoxycytidine, and deoxythymidine in three lengths (5-, 14-, and 28-mer). An N^3-methylthymine-containing analog of *S*-ODN-1 (N-Me-*S*-ODN-1) was used to assess the role of hybridization in the antiviral effect, since N^3-methyl substitution on thymidine is known to significantly reduce duplex stability (14). In fact, N-Me-*S*-ODN-1 gave no measurable melting temperature (midpoint of duplex to single-strand transition; t_m) with its complementary DNA sequence under physiological conditions (unpublished data).

Cytopathic Effect Inhibition Assay for Assessment of Anti-HIV Activity. We previously reported an *in vitro* HIV cytopathic effect inhibition assay for assessing anti-HIV activity (15). In this *in vitro* assay system, immortalized T4$^+$ T cells (ATH8 cells) were used as target cells because of their profound sensitivity to the cytopathic effect of HIV. The target cells (2×10^5 ATH8 cells) in each tube were pretreated with the stated concentration of each oligomer for 16 hr (although it was subsequently found this pretreatment was

Abbreviations: ddAdo, 2′,3′-dideoxyadenosine; ddCyd, 2′,3′-dideoxycytidine; HIV, human immunodeficiency virus; HTLV-III, human T-lymphotropic virus type III; IL-2, interleukin 2; ODN, oligodeoxynucleotide; *M*-ODN, methylphosphonate ODN; N-Me-ODN, N^3-methylthymidine-containing ODN; n-ODN, normal ODN; *S*-ODN, phosphorothioate ODN; t_m, melting temperature (midpoint of duplex to single-strand transition).

§Present address: Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404.

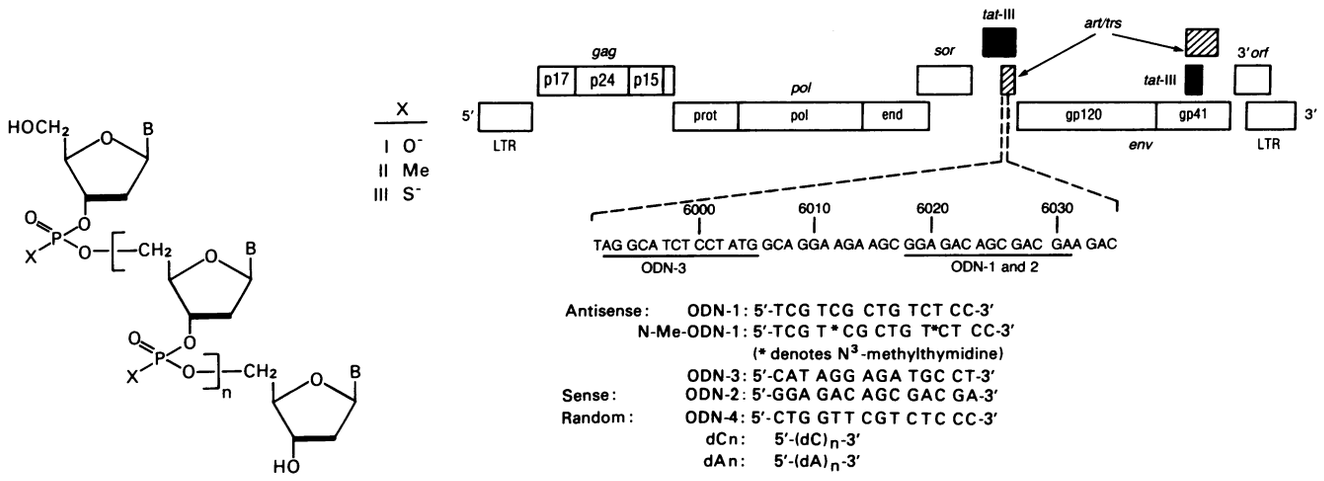

FIG. 1. (*Left*) General molecular structure of normal (I), methylphosphonate (II), and phosphorothioate (III) ODNs (n-ODN, *M*-ODN, and *S*-ODN, respectively). B is adenine, guanine, cytosine, or thymine. (*Right*) DNA sequence of coding exon I of *art/trs* gene in human T-cell lymphotropic virus type III [now called human immunodeficiency virus (HIV)] (HTLV-III) BH10 and sequences of ODNs tested. N-*Me*-ODN-1 is N^3-methyl-thymidine-containing antisense ODN, which has methylated thymidine at positions 4 and 9. Random sequence ODN-4 has a same base content as ODN-1 but has <70% homology with any sequence in HTLV-III BH10 genomic sequence as antisense or sense. Homooligomers of deoxycytidine and deoxyadenosine were synthesized with three lengths (5-, 14-, and 28-mer). LTR, long terminal repeat.

unnecessary) and then incubated with Polybrene for 45 min. Following the centrifugation, each set of pelleted cells was incubated with HTLV-III_B for 1 hr [500 virus particles per cell unless otherwise indicated; this dose is ≈100–1000 times higher than the minimum cytopathic dose in our assay system (16)]. Complete medium (2 ml of RPMI 1640) supplemented with L-glutamine (4 mM), 2-mercaptoethanol (50 nM), penicillin (50 units/ml), and streptomycin (50 μg/ml) and containing 15% fetal calf serum and interleukin 2 (IL-2) (15% of conventional IL-2 from Advanced Biotechnologies, Silver Spring, MD, and 20 units of recombinant IL-2 per ml from Amgen Biologicals, Thousand Oaks, CA) was used with various concentrations of oligomers added. The number of viable cells was counted in a hemocytometer using the trypan blue dye-exclusion method on day 7 following exposure to the virus. Each set of data in tables and figures was obtained from simultaneously performed experiments to make a precise comparison among agents tested.

Determination of HIV *gag*-Encoded Protein Expression. The percentage of the cells expressing p24 *gag* (group-specific antigen) protein of HIV was determined by indirect immunofluorescence microscopy as described (17) by using anti-HIV p24 murine monoclonal antibody (M26; kindly provided by F. D. Veronese and R. C. Gallo).

Southern Blot Analysis. Target cells (1×10^7 ATH8 cells) were pretreated with or without a 28-mer phosphorothioate oligodeoxycytidine (*S*-dC_{28}) at various concentrations for 16 hr, treated with Polybrene, exposed to HTLV-III_B (500 virus particles per cell), resuspended, and cultured in the presence or absence of *S*-dC_{28}. On days 4 and 7 following exposure to the virus, high molecular weight DNA was extracted, digested with Asp718 (a *Kpn* I isoschizomer) (Boehringer Mannheim), and subjected to Southern blot analysis hybridized with a labeled insert of molecular clone of the *env* region of HTLV-III (BH10) containing a 1.3-kilobase (kb) *Bgl* II fragment.

RESULTS

Antiviral Activity of Phosphorothioate Analogs. Results of the antiviral effect and cytotoxicity of ODNs are presented in Table 1. The two n-ODNs and one *M*-ODN tested showed no significant inhibitory effects, whereas all of the *S*-ODNs exhibited significant inhibition of the cytopathic effect of HIV. Surprisingly, the 14-mer phosphorothioate homooligomer of dC(*S*-dC_{14}) was found to be the most potent antiviral compound among those tested in this series of experiments. Since phosphorothioate ODNs that are not antisense sequences appear to be very effective antiviral agents, we tried to clarify the nature of the base composition effect. When comparing the effects of each of the 14-mer phosphorothioates tested at 5 μM, it was found that inhibition of the viral cytopathic effect was approximately linear with respect to the G+C content of the analog (data from Table 1).

Table 1. Antiviral effect and cytotoxicity of ODNs

	Antiviral effect,* %				Cytotoxicity,† %			
Compound‡	1 μM	5 μM	10 μM	25 μM	1 μM	5 μM	10 μM	25 μM
S-ODN-1	0	43	72	95	0	0	0	20
n-ODN-1	3	2	9	4	35	22	27	14
M-ODN-1	8	20	13	10	20	27	20	20
S-ODN-2	11	56	100	78	0	0	0	6
n-ODN-2	11	9	0	11	18	28	35	32
S-ODN-3	0	6	44	94	0	0	0	13
S-ODN-4	0	53	78	100	0	0	0	0
S-dC_{14}	25	100	100	100	0	0	0	0

Experiments were performed simultaneously and were duplicated except for *M*-ODN-1 (see legend of Fig. 2 for the detailed method).

*The percentage of protective effect of ODN on survival and growth of target cells (ATH8 cells) exposed to the virus was determined by the following formula: 100 × [(number of total viable cells exposed to HTLV-III_B and cultured in the presence of ODN − number of total viable cells exposed to HTLV-III_B and cultured in the absence of ODN)/(number of total viable cells cultured alone − number of total viable cells exposed to HTLV-III_B and cultured in the absence of ODN)]. By this formula, calculated percentage ≥ 100 is expressed as 100%, which represents complete protective effect without cytotoxicity to target cells.

†The percentage of cytotoxicity of ODN on the growth of target cells (ATH8 cells) was determined by the following formula: 100 × [(1 − number of total viable cells cultured in the presence of ODN)/number of total viable cells cultured alone]. Calculated percentages ≤ 0 are expressed as 0%.

‡*S*, ODN phosphorothioate analog of given sequence; n, unmodified (normal) ODN of given sequence; *M*, ODN methylphosphonate analog of given sequence.

Comparison of Anti-HIV Activity in Various Lengths of Oligo(dC) and Oligo(dA) Phosphorothioates. Because it is possible that interassay variation may create an inappropriate comparison of antiviral activity among agents, we performed experiments simultaneously to make more precise comparisons.

As illustrated in Fig. 2 *Left*, the inhibitory effects of *S*-dC_n are greater and more persistent than those of *S*-dA_n for the 14-mer and 28-mer, whereas 5-mers belonging to both categories fail to significantly inhibit the cytopathic effect of the virus. The order of effectiveness of the homooligomers was deoxycytidine > deoxythymidine ≥ deoxyadenosine for the 14-mer (data not shown for deoxythymidine). We then explored the effect of oligomer length in detail. The longer sequences were found to be more effective than the shorter sequences at the same molar concentration of nucleotide unit. For example, in Fig. 2 *Right*, the 28-mer *S*-dC_{28} at concentrations as low as 0.5 μM (13.5 μM nucleotide equivalents) gave complete protection against the virus, whereas the corresponding 14-mer at 5 μM (65 μM equivalents) had only a moderate effect. The *S*-dC_{28} gave the most consistent and durable antiviral effects under the conditions used in these experiments. These data suggest a real *length effect* and argue against either metal ion chelation or degradation to reactive monomers.

Effect of N^3-methylthymidine Substitution in ODN Analog on Anti-HIV Activity. N^3-methylthymidine-containing *S*-ODN-1 showed no anti-HIV activity, whereas *S*-ODN-1 consistently exhibited substantial activity against HIV (Fig. 3). Since N^3-methyl substitution on the pyrimidine base is known to profoundly reduce hydrogen bonding to complementary adenosine residues (14), the relative inactivity of this N^3-methylthymine-containing analog of phosphorothioate suggests that antiviral activity could be brought about by binding to nucleotide sequences as at least one mechanism.

Inhibition of *de Novo* HIV DNA Synthesis in ATH8 Cells Exposed to the Virus by the 28-mer of Oligodeoxycytidine Phosphorothioate. Fig. 4 shows the inhibitory effect of the Phosphorothioate oligodeoxycytidine analog (*S*-dC_{28}) on *de novo* HIV DNA synthesis in target cells. On days 4 and 7 following exposure to the virus, a substantial amount of viral DNA was detected by Southern blot analysis without antiviral agents. *S*-dC_{28}, as well as ddAdo as the positive control, significantly inhibited the *de novo* synthesis of viral DNA at concentrations as low as 1 μM.

Failure to Inhibit Expression of Viral Protein by 28-mer Oligodeoxycytidine Phosphorothioate (*S*-dC_{28}) in Chronically HIV-Infected T Cells. As illustrated in Table 2, *S*-dC_{28} failed to reduce *gag* protein positivity of target cells assessed by indirect immunofluorescent assay in chronically HIV-infected H9 cells at concentrations as high as 25 μM for the duration of the experiment (120 hr).

Synergistic Enhancement of Antiviral Activity of ddAdo by 14-mer Oligodeoxycytidine Phosphorothioate. It is worth stressing that the various dideoxynucleosides [including azidothymidine, 2',3'-dideoxycytidine (ddCyd), and ddAdo] require anabolic phosphorylation within target cells to become active antiretroviral agents. The mechanisms of action appear to be competitive inhibition of reverse transcriptase and/or termination of nascent DNA chain formation. The oligomers under discussion are likely to work by different mechanisms and would not be expected to require anabolic

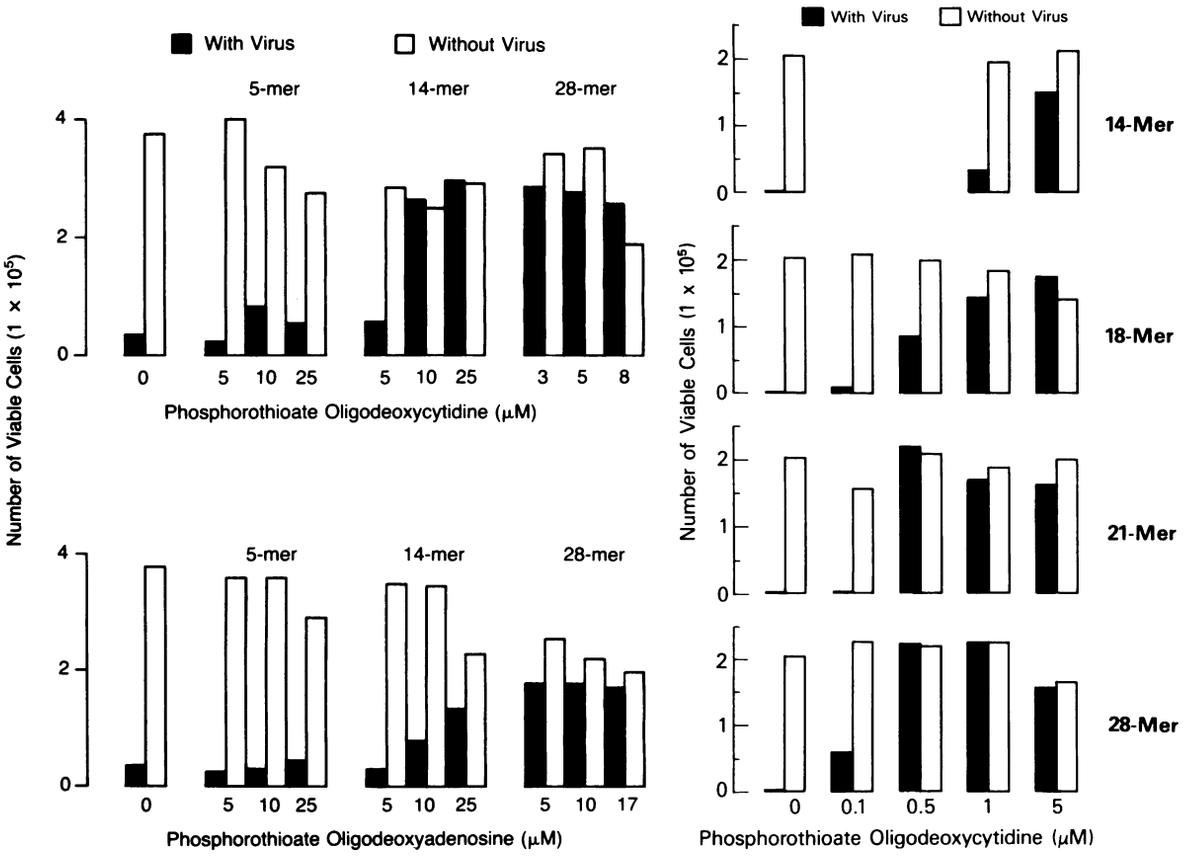

FIG. 2. (*Left*) Comparison of anti-HIV activity in three lengths of *S*-dCs and *S*-dAs. Filled columns represent virus-exposed cells and open columns represent non-virus-exposed cells. The inhibitory effects of *S*-dC_n are greater and more persistent than those of *S*-dA_n for the 14-mer and 28-mer. The longer sequences were found to be more effective than the shorter ones. (*Right*) Detailed comparison of anti-HIV activity between the 14-mer and 28-mer of *S*-dC_n. There is an obvious *length effect* even with an increase of several nucleotide lengths as short as three nucleotides.

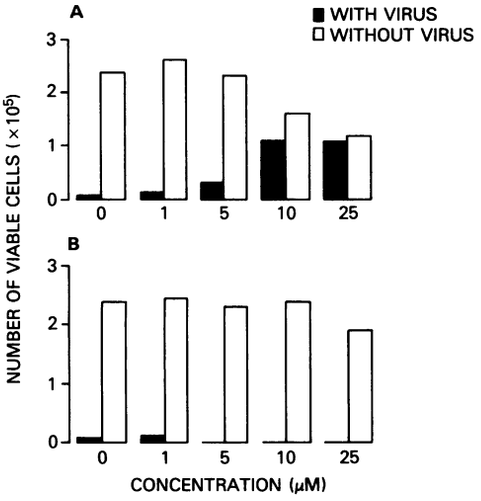

FIG. 3. Effect of *N*-methylation of thymine on antiviral activity of *S*-ODN-1. (*A*) *S*-ODN-1 showed antiviral activity at 10 and 25 μM. (*B*) N^3-methylthymidine-containing *S*-ODN-1 failed to show activity at 1, 5, 10, and 25 μM.

phosphorylation. It was, therefore, of interest to see if we could observe synergistic antiviral effects when we combined an antiviral oligomer with a dideoxynucleoside. The combination of ddAdo and 14-mer oligodeoxycytidine phosphorothioate gave a marked synergistic enhancement of antiviral activity (Fig. 5). For example, 2 μM ddAdo showed complete protection of target cells against the viral cytopathic effect with 5 μM *S*-dC_{14}, whereas each of the agents alone showed very marginal protective effects in this experiment.

DISCUSSION

Since only phosphorothioate analogs showed anti-HIV activity (Table 1) in our assay, we presume that it is mainly the relative resistance of the phosphorothioate analogs to nucleases that preserves them relative to n-ODNs and allows them to reach and remain at their target site. This was supported in relation to the medium used in our *in vitro* test system by following the ^{31}P NMR spectra of the n-ODN-1 and *S*-OND-1 compounds as a function of time. Breakdown of the n-ODN was seen from the buildup of the terminal phosphate peak, indicating a half-life of ≈17 hr under these conditions,

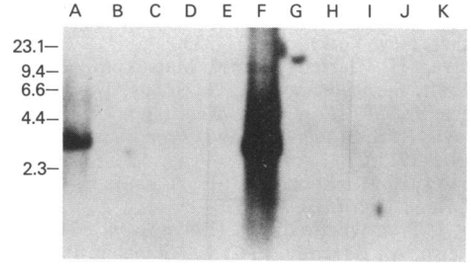

FIG. 4. Inhibition of *de novo* HIV DNA synthesis in ATH8 cells exposed to the virus by the 28-mer of oligodeoxycytidine phosphorothioate. On day 4 (lanes A–E) and day 7 (lanes F–J) following exposure to the virus, high molecular weight DNA was extracted. Lanes A and F contain DNA from ATH8 cells that were exposed to the virus and not protected by *S*-dC_{28}. Lanes B and G, C and H, and D and I contain DNA from ATH8 cells pretreated and cultured with 1, 5, and 7 μM *S*-dC_{28}, respectively. Lanes E and J contain DNA from ATH8 cells treated with 50 μM 2′,3′-dideoxyadenosine (ddAdo), and lane K contains DNA from ATH8 cells that was not exposed to the virus. Sizes are shown in kb. The 2.7-kb *env*-containing internal *Kpn* I fragment of the virus genome was detected only in lanes A and F.

Table 2. Failure to reduce the positivity of *gag* protein by 28-mer phosphorothioate analogs in chronically HTLV-III_B-infected H9 (H9/III_B) cells

S-dC_{28}, μM	% of *gag*-positive cells			
	8 hr	24 hr	72 hr	120 hr
0	79	90	70	78
5	82	91	85	79
10	74	80	71	82
25	69	86	75	74

We used chronically HTLV-III_B-infected (>6 months) to test the inhibitory effect of phosphorothioate analogs on expression of viral gene. At 8, 24, 72, and 120 hr in culture in the presence or absence of phosphorothioate analogs following exposure to compounds, H9/III_B cells were washed three times with phosphate-buffered saline and subjected to indirect immunofluorescence assay using monoclonal anti-p24 antibody. The 28-mer oligodeoxycytidine phosphorothioate did not reduce *gag* protein positivity of chronically infected H9 cells.

whereas the *S*-analogs exhibited no significant degradation even after a week (within the accuracy of the method, <5%). Similarly, samples of solutions of *S*-ODNs taken from our *in vitro* cytopathic assay and incubated in human serum at 37°C showed no degradation after 7 days (data not shown). The inactivity of a methylphosphonate analog (*M*-ODN-1) in our cytopathic inhibition assay could have been due to its poor

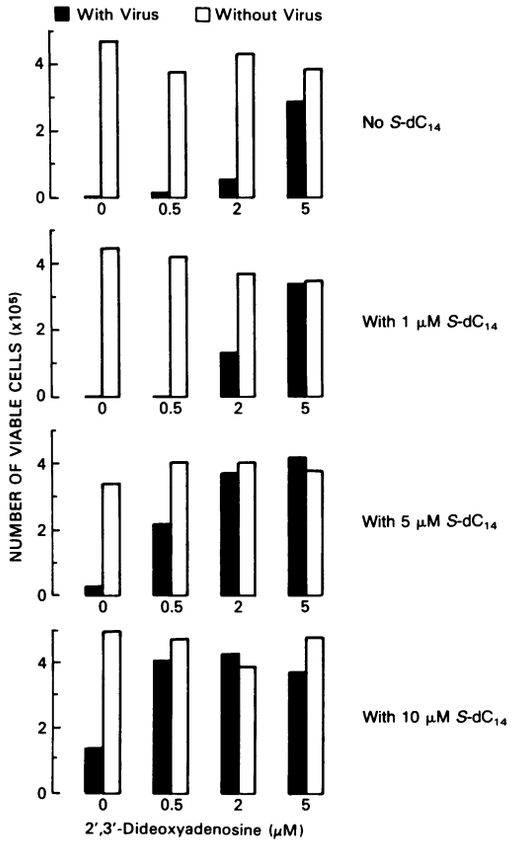

FIG. 5. Synergistic enhancement of antiviral activity of ddAdo with *S*-dC_{14}. The target cells were pretreated with various concentrations of *S*-dC_{14} for 16 hr, pretreated with Polybrene (2 μg/ml), exposed to 1000 virus particles per cell for 1 hr, and resuspended in 2 ml of complete medium containing IL-2 with or without various concentrations of ddAdo. On day 13 after exposure to the virus, viable cells were counted by the trypan blue dye-exclusion method. These experiments involved a more potent viral inoculum for a longer duration than in the other experiments reported here.

ability to hybridize strongly to the target sequence (low t_m; unpublished data).

The potency of anti-HIV activity of *S*-dC_{28}, one of most potent analogs tested, is almost comparable to that of ddCyd (15) on the basis of molarity (both agents showed complete antiviral activity at 0.5 μM in our assay system); also in terms of therapeutic index (ratio of cytotoxic concentration to effective concentration), *S*-dC_{28} generally shows a comparable *in vitro* index to those of ddCyd and ddAdo (i.e., 10–20) (15).

Generally it has been assumed that antisense sequences inhibit the expression of various genes by translation arrest—i.e., that they bind to mRNA and block its translation (18, 19). To test this possibility we analyzed *gag* protein synthesis in chronically HTLV-III_B-infected and -producing H9 (H9/III_B) cells by indirect immunofluorescent assay under a microscope. *S*-dC_{28} did not inhibit *gag* protein positivity in H9/III_B cells at concentrations as high as 25 μM (Table 2). Although *gag* positivity of cells is only a partially quantitative parameter for protein production, this result suggests that the potent anti-HIV activity of *S*-dC_{28} at concentration as low as 0.5 μM might not be from a translation arrest *per se*. Alternatively, the level of any translation arrest could have been below our threshold of detection by indirect immunofluorescent assay under a microscope. By contrast, a Southern blot analysis employed to explore *de novo* synthesis of HIV DNA in target cells showed complete inhibition by *S*-dC_{28} at concentrations down to 1 μM (Fig. 4). Therefore, one mechanism for the antiviral effect could depend on blocking viral replication perhaps prior to, or at the stage of, proviral DNA synthesis.

We tested the possibility that the *S*-ODN analogs may interfere with HIV binding to target cells. The T4 molecule on the cell surface is known to be the main receptor for HIV in $T4^+$ cells (20). No inhibition by *S*-dC_{28} was observed in experiments using radiolabeled virus for specific binding of the labeled virus to the T4 molecule in $T4^+$ cells (H9 cells), thus suggesting that inhibition of viral binding to the cell surface is not responsible for the activity (D. Looney, personal communication). In addition, no detectable changes in the T4, HLA-DR, T8, T3, or Tac antigen on the cell surface of ATH8 cells were shown by fluorescent-activated cytofluorometry after 16 hr of incubation with 1 μM *S*-dC_{28} (data not shown). Overall these findings, including a *base composition effect* and a *length effect* (Table 1 and Fig. 2 *Left* and *Right*), suggest that the antiviral activity is mediated by inhibition of HIV proviral DNA synthesis, perhaps brought about, at least in part, by binding of the *S*-ODNs to a viral nucleotide sequence.

Another mechanism to be considered is induction of interferon production such as that proposed for phosphorothioate analogs of poly-r(I-C) (21). No induction of γ-interferon was observed in the supernatant of the culture with *S*-dC_{14}, and 1000 units of recombinant α- or γ-interferon added directly to the cultures did not inhibit the cytopathic effect in our assay system. Also since there are no data to support the concept that phosphorothioate internucleotide linkages have a thiol character, and can thus form disulfides, the mechanism of action would likely be different from that proposed for antiviral polynucleotides having thiolated bases such as 5-mercaptocytosine or -uracil (22).

Phosphatase-resistant ^{35}S-labeled phosphorothioate (end-labeled) (23) *S*-dC_{28} was employed to investigate the permeability of target cells. Significant increases of radioactivity in ATH8 and H9 cells were observed within several minutes (unpublished data), as reported for n-ODNs (3), thus supporting the uptake of these compounds by the cells.

S-ODNs also showed substantial inhibition of purified HIV reverse transcriptase activity in the *in vitro* experiment using a viral DNA (3′ *orf*) inserted in an M13 vector (16) as a template with universal primer (unpublished data) under previously described conditions (24). Under some conditions, phosphorothioate analogs can serve as competitive inhibitors of template-primer (unpublished data), and this class of compounds appears to have multiple mechanisms of action. The precise mechanism(s), however, including nonsequence specificity of the antiviral activity, direct inhibition of the viral DNA polymerase, or additional translation arrest at high concentration for complementary sequences requires further research at this time. Nevertheless, the anti-HIV effect of these agents is potentially important from a clinical and theoretical point of view.

We thank Flossie Wong-Staal for helpful advice and M. G. Sarngadharan and Charles Myers for helpful discussion. We also thank C. F. Perno for a measurement of γ-interferon. This work was supported in part by U.S. Army Medical Research and Development Command Grant Y03-CM-70147 to J.S.C.

1. Zamecnik, P. C. & Stephenson, M. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 280–284.
2. Stephenson, M. L. & Zamecnik, P. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 285–288.
3. Zamecnik, P. C., Goodchild, J., Taguchi, Y. & Sarin, P. S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4143–4146.
4. Wickstrom, E. (1986) *J. Biochem. Biophys. Methods* **13**, 97–102.
5. Smith, C. C., Aurelian, L., Reddy, M. P., Miller, P. S. & Ts'o, P. O. P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2787–2791.
6. Miller, P. S., Reddy, M. P., Murakami, A., Blake, K. R., Lin, S. B. & Agris, C. H. (1986) *Biochemistry* **25**, 5092–5097.
7. De Clercq, E. D., Eckstein, F., Sternbach, H. & Merigan, T. C. (1970) *Virology* **42**, 421–428.
8. Stec, W. J., Zon, G., Ega, W. & Stec, B. (1984) *J. Am. Chem. Soc.* **106**, 6077–6089.
9. Tanabe, T. & Yamaguchi, K. (1979) *Bull. Chem. Soc. Jpn.* **52**, 204–207.
10. Arya, S. K., Guo, C., Josephs, S. F. & Wong-Staal, F. (1985) *Science* **229**, 69–73.
11. Dayton, A. I., Sodroski, J. G., Rosen, C. A., Goh, W. C. & Haseltine, W. A. (1986) *Cell* **44**, 941–947.
12. Feinberg, M. B., Jarrett, R. F., Aldovini, A., Gallo, R. C. & Wong-Staal, F. (1986) *Cell* **46**, 807–817.
13. Sodroski, J., Goh, W. C., Rosen, C., Dayton, A., Terwilliger, E. & Haseltine, W. (1986) *Nature (London)* **321**, 412–417.
14. Kyogoku, Y., Lord, R. C. & Rich, A. (1967) *Proc. Natl. Acad. Sci. USA* **57**, 250–257.
15. Mitsuya, H., Matsukura, M. & Broder, S. (1987) in *AIDS: Modern Concepts and Therapeutic Challenges*, ed. Broder, S. (Dekker, New York), pp. 303–333.
16. Mitsuya, H., Jarrett, R. F., Matsukura, M., Veronese, F. D. M., Sarngadharan, M. G., Johns, D. G., Reitz, M. & Broder, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2033–2037.
17. Mitsuya, H. & Broder, S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1911–1915.
18. Paterson, B. M., Roberts, B. E. & Kuff, E. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4370–4374.
19. Izant, J. T. & Weintraub, H. (1985) *Science* **229**, 345–352.
20. Dalgleish, A., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984) *Nature (London)* **312**, 763–767.
21. LaPlanche, L., James, T. L., Powell, C., Wilson, W. D., Uznanski, B., Stec, W. J., Summers, M. F. & Zon, G. (1986) *Nucleic Acids Res.* **14**, 9081–9093.
22. Aradi, J. & Ho, Y. K. (1985) *Cancer Biochem. Biophys.* **7**, 349–359.
23. Eckstein, F. (1985) *Annu. Rev. Biochem.* **54**, 367–402.
24. Veronese, F. D. M., Copeland, T. D., Devico, A. L., Rahman, R., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. (1986) *Science* **231**, 1289–1291.