

Inhibition of human immunodeficiency virus in early infected and chronically infected cells by antisense oligodeoxynucleotides and their phosphorothioate analogues

(antiviral drugs/hybridization arrest)

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ABSTRACT Antisense oligodeoxynucleotides, both the phosphorothioate analogues and unmodified oligomers of the same sequence, inhibit replication and expression of human immunodeficiency virus already growing in tissue cultures of MOLT-3 cells with much greater efficacy than do mismatched ("random") oligomers and homooligomers of the same length and with the same internucleotide modification. This preferential inhibitory effect is elicited in as short a time as 4–24 hr postinfection. Likewise, antisense oligomers exhibit greater inhibitory effects on human immunodeficiency virus in chronically infected cells than do mismatched oligomers and homooligomers. Phosphorothioate antisense oligomers are up to 100 times more potent than unmodified oligomers of the same sequence in these inhibitory assays. These results, in major respects, confirm and extend those recently published by Matsukura *et al.* [Matsukura, M., Zon, G., Shinozuka, K., Robert-Guroff, M., Shimada, T., Stein, C. A., Mitsuzaka, H., Wong-Staal, F., Cohen, J. S. & Broder, S. (1989) *Proc. Natl. Acad. Sci. USA* 86, 4244–4248]. They also point out the importance of computer analysis of sequences thought to be random but that in reality contain significant areas of likely hybridization, either to the viral genome or to the complementary DNA strand synthesized from it. They thus reinforce the concept that specific base pairing is a crucial feature of oligonucleotide inhibition of human immunodeficiency virus.

Antisense oligodeoxynucleotides and their analogues have been used as tools for inhibiting viral replication (1–3), for blocking splicing and translation of mRNA (4, 5), and for regulating specific gene expression (6, 7). It has been found that an oligonucleotide complementary to a segment of a viral genome or an mRNA derived therefrom may interfere with the expression of that genetic segment by hybridization competition (1, 2).

In earlier reports, we demonstrated that replication of human immunodeficiency virus (HIV) could be inhibited by normal phosphodiester oligodeoxynucleotide sequences complementary to HIV RNA (4, 8). However, relatively short half-lives of normal oligonucleotides (9) in serum and in cells due to the presence of nucleases, and possibly the low permeability of these charged molecules into normal cells, limit their potential usefulness *in vivo*. To overcome this limitation, we and others (10–15) have studied internucleotide phosphate backbone-modified oligonucleotides, such as methylphosphonates (3, 10) as well as phosphorothioates and various phosphoramidates (11, 12), for their antiviral activities against HIV.

Here we report both the inhibition of HIV replication and the effect on cell growth of MOLT-3 cells that have been infected just 24 or 48 hr before addition of oligomers and also the effect on MOLT-3 cells chronically infected with HIV that were mixed with uninfected cells in the presence of oligodeoxynucleotides and their phosphorothioate analogues.

MATERIALS AND METHODS

Oligonucleotide Synthesis. Oligonucleotides were synthesized on an automated synthesizer (model 8700, Milligen Biosearch, Bedford, MA). Both normal phosphodiester oligonucleotides and their phosphorothioate analogues were assembled by using H-phosphonate chemistry (11, 16, 17). Synthesis was carried out on a 15- to 25- μ mol scale on controlled pore glass support (loading of nucleoside = 35–40 μ mol/g). For each coupling reaction, a 2- to 3-fold molar excess of nucleoside H-phosphonate and a 20- to 30-fold molar excess of adamantanecarbonyl chloride in acetonitrile/pyridine (1:1, vol/vol) were used for 60–80 sec, and 3% dichloroacetic acid in dichloroethane for 90–120 sec was used for each detritylation step. After the chain elongation cycles, the controlled pore glass support-bound oligonucleoside H-phosphonate was either treated with 2% iodine in pyridine/H₂O (98:2, vol/vol) to generate the phosphodiester linkage or with 0.2 M sulfur in carbon disulfide/triethylamine (9:1, vol/vol) (11) or 0.2 M sulfur in a mixture of carbon disulfide/pyridine/triethylamine (12:12:1, vol/vol) at room temperature for up to 2 hr to obtain the phosphorothioate internucleotide linkage. Deprotection of oligonucleotide was carried out with concentrated ammonia at 55°C for 8 hr. Deprotected oligonucleotides were purified by low-pressure reverse-phase chromatography (preparative C₁₈, 55–105 μ m; Waters) with a linear gradient of acetonitrile (0–80% for 80 min) in 0.1 M ammonium acetate (pH 7.0). Purification was also carried out by low-pressure ion-exchange chromatography on DEAE-cellulose (DE-52, Whatman) by using a linear gradient of triethylammonium bicarbonate at pH 8 (0.01–2 M triethylammonium bicarbonate over 90 min). After purification on reverse-phase and ion-exchange columns, oligonucleotides were dialyzed against double-distilled H₂O for 48 hr (wet cellulose dialysis tubing, molecular weight cutoff of 3000; Spectrum Medical Industries). After dialysis, oligonucleotides were lyophilized. Finally, oligonucleotides were characterized by HPLC, polyacrylamide gel electrophoresis, base composition, thermal stability of duplexes, UV absorbance, and NMR spectroscopy. Phosphorothioate oligonucleotides were used as a mixture of stereoisomers at phosphorus for biological experiments.

Abbreviation: HIV, human immunodeficiency virus.

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Large-scale preparation of oligonucleotides 1 and 3 for rat toxicity studies was carried out on a 6 × 20-μmol scale on an automated synthesizer.

Assay for HIV-1 Inhibition. Inhibition of HIV-1 expression in H9 or MOLT-3 cells in the presence of oligonucleotides was carried out as reported earlier (10, 11, 18, 19). Antisense oligomers were added at different concentrations only once, either at 0 hr (within a few seconds of virus addition) or 24 or 48 hr after addition of virus to the cells. The cells were incubated in culture medium in a humidified atmosphere containing 5% CO₂ at 37°C. After 4 days the cells and supernatant were examined for the level of HIV-1 expression by measuring syncytia (MOLT-3 cells), the expression of viral antigens p24 and p17, reverse transcriptase activity, as well as cell viability, as reported earlier (10, 11, 18, 19). Twenty-four hours after viral infection, the cells were washed, then the oligomer was added, and the experiment was carried on for 4 days thereafter before termination. When oligomer was added similarly 48 hr after viral infection, the experiment was likewise terminated 4 days after oligomer addition.

To study the inhibition of HIV-1 expression by oligodeoxynucleotide in chronically infected cells, one part of MOLT-3 cells chronically infected with HIV-1 (HTLV-III_B) was mixed with four parts of uninfected MOLT-3 cells and incubated with different concentrations of oligonucleotides for 4 days. HIV-1 expression was measured by syncytia, p17, p24, and reverse transcriptase assays as described above.

RESULTS

In the present study, four oligonucleotides were tested, and details are given in Tables 1 and 2. Oligonucleotide 1 is complementary to the splice acceptor site at nucleotides 5349–5368 in HIV (20) and has been shown to inhibit replication of this virus in MOLT-3 cells (4). Oligonucleotide 2 was originally chosen as a mismatched sequence (“random”) control for comparison with oligonucleotide 1. Likewise, oligonucleotide 4 is the phosphorothioate analogue of oligonucleotide 2. In separate experiments, phosphorothioate analogues of dT₂₀ and dA₂₀ were also tested.

Comparative Anti-HIV Activity of Oligonucleotides 1 and 3. Oligonucleotides 1 and 3 were tested for their comparative anti-HIV activity in one experiment. Oligonucleotide and virus particles of HIV-1 were added to the cell culture at the same time. The inhibition of HIV expression was monitored

Table 2. Inhibition of HIV-1 expression by oligodeoxynucleotides in a mixture of chronically infected cells and uninfected cells

Oligo-nucleotide no.	Sequence	Conc., μg/ml	% inhibition			
			Syn.	p17	p24	RT
<i>Phosphodiesteres</i>						
1	Antisense	12.5	5	17	21	20
		50	29	37	33	30
		200	54	48	42	50
2	Mismatched	12.5	10	13	8	7
		50	14	17	13	12
		200	49	39	46	36
<i>Phosphorothioates</i>						
3	Antisense	1	16	10	10	31
		5	43	35	38	51
		10	76	61	72	63
4	Mismatched	1	7	0	0	17
		5	32	32	25	33
		10	48	31	33	35

MOLT-3 cells chronically infected with HTLV-III_B (one part) were washed, then mixed with uninfected MOLT-3 cells (four parts), and incubated with different concentrations of oligonucleotide for 4 days. Syn., syncytia; RT, reverse transcriptase.

by syncytial formation, viral antigen p17 and p24 expression, and reverse transcriptase activity. Both oligonucleotides gave dose-dependent inhibition of HIV-1 expression as assayed by the above-mentioned four parameters (Fig. 1). Oligonucleotide 1 showed 80–90% inhibition at a dose of 200 μg/ml, whereas oligonucleotide 3 showed similar activity at 2 μg/ml (approximately a 100-fold greater inhibitory potency).

Anti-HIV Activity of Oligonucleotides 1 and 2 (Phosphodiester Oligomers). Data on inhibition of formation of syncytia, expression of HIV proteins p17 and p24, and reverse transcriptase activity are given in Table 1. In three separate sets of experiments, oligomers were added to HIV-infected cells (i) at 0 hr (simultaneously with virus), (ii) after 24 hr of infection, and (iii) after 48 hr of infection. The oligonucleotides were added only once. Oligonucleotide 1 (antisense oligomer) at 0 hr of addition gave dose-dependent inhibition of HIV-1 expression as assayed by the above-mentioned four parameters. At 200 μg/ml (33 μM), inhibition was 80–90%.

Oligonucleotide 2 also showed similar inhibition when added at 0 hr. When the same concentrations of oligonucle-

Table 1. Inhibition of HIV-1 expression by oligodeoxynucleotides

Oligonucleotide No.	Sequence	HIV binding site	Conc. μg/ml	μM	% inhibition											
					0 hr*				24 hr*				48 hr*			
Syn.	p17	p24	RT	Syn.	p17	p24	RT	Syn.	p17	p24	RT	Syn.	p17	p24	RT	
<i>Phosphodiesteres</i>																
1	ACACCCAATTCTGAAAATGG	5349–5368	12.5	2	20	19	17	35	0	6	3	0	0	0	0	0
			50	8	80	59	61	65	0	9	3	0	0	0	0	0
			200	33	85	84	89	83	10	9	13	27	0	0	0	0
2	GCAGGCAAACCATTTGAATG	?	12.5	2	16	22	14	35	0	0	0	0	0	0	0	0
			50	8	58	29	17	46	0	0	0	0	0	0	0	0
			200	33	96	83	70	84	0	0	0	0	0	0	0	0
<i>Phosphorothioates</i>																
3	ACACCAATTCTGAAAATGG	5349–5368	1	0.1	0	0	0	0	0	0	0	0	0	0	0	0
			5	0.8	96	83	87	68	33	18	27	35	0	3	3	17
			10	1.6	100	96	96	83	91	88	84	93	54	36	32	44
4	GCAGGCAAACCATTTGAATG	?	1	0.1	0	0	0	0	0	0	0	0	0	0	0	0
			5	0.8	54	31	36	58	0	3	3	0	0	0	0	0
			10	1.6	98	87	83	69	0	0	0	0	0	0	0	0

Syn, syncytia; RT, reverse transcriptase; ?, sequence originally considered to be a random control but that possesses significant binding stretches to the HIV genome.

*The oligonucleotides were added 0, 24, or 48 hr after infection of MOLT-3 cells with HIV-1 (HTLV-III_{CC}).

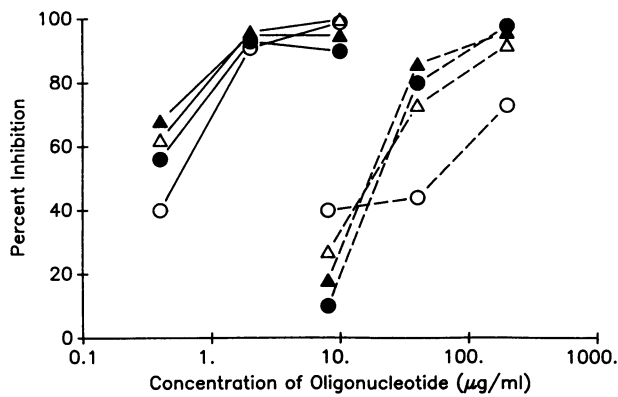


FIG. 1. Comparative percent inhibition of HIV-1 expression by unmodified oligonucleotide 1 (---) and phosphorothioate oligonucleotide 3 (—). Both oligonucleotides are identical in sequence and complementary to the splice acceptor site (5349–5368) of HIV. Concentration of oligonucleotides is plotted as the logarithm of the concentration (in $\mu\text{g}/\text{ml}$). HIV expression was assayed by inhibition of syncytia formation (Δ), p17 (\blacktriangle) and p24 expression (\circ), and reverse transcriptase activity (\bullet). HIV-1 expression assayed by any of the four assay systems gave similar results.

otide 1 were added to cells that were infected for 24 hr, only 10–30% inhibition was obtained at a concentration of 200 $\mu\text{g}/\text{ml}$ (Fig. 2). No inhibition was noticed when oligonucleotides 1 and 2 were added after 48 hr of infection.

Oligonucleotides 1 and 2 were also tested for their antiviral activity against a mixture of one part of chronically infected cells and four parts of uninfected cells (Table 2). Inhibition of HIV-1 expression was dose-dependent for oligonucleotides 1 and 2, and 40–55% inhibition was obtained at a concentration of 200 $\mu\text{g}/\text{ml}$. It should be noted that a comparable degree of inhibition was obtained by the phosphorothioate oligomers at a much lower dosage (Table 2).

Effect of Oligodeoxynucleotides 1 and 2 on Growth of HIV-Infected Cells. The results are summarized in Fig. 3. Oligonucleotides 1 and 2 permitted normal growth of HIV-infected cells when added at zero time; however, there was no dose dependence. At a concentration of 6.25 $\mu\text{g}/\text{ml}$, the same effect was noticed as at a concentration of 100 $\mu\text{g}/\text{ml}$. When the same concentration of oligonucleotides was added at 24 or 48 hr after infection, there was no sign of improvement on HIV-infected cell growth.

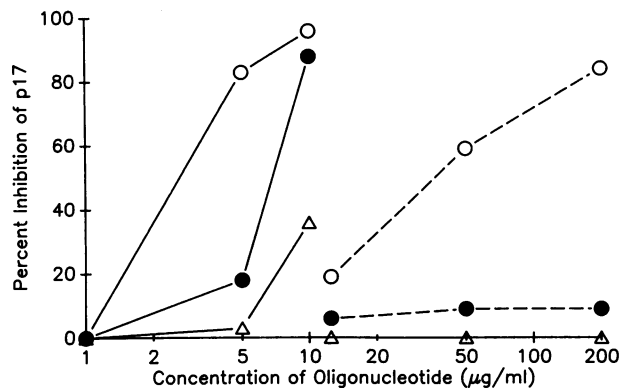


FIG. 2. Comparative percent inhibition of HIV expression as measured by p17 protein expression by oligonucleotide 1 (---) and oligonucleotide 3 (—), when they were added to cell cultures at 0 hr (\circ), 24 hr (\bullet), or 48 hr (Δ) after infection. Concentration of oligonucleotide is plotted as the logarithm of the concentration (in $\mu\text{g}/\text{ml}$). The inhibition of HIV-1 expression is dose dependent even if oligonucleotide 3 is added 0, 24, or 48 hr after infection, but percent inhibition goes down at the same dose with time of infection. Oligonucleotide 1 showed no inhibition after 24 hr of infection.

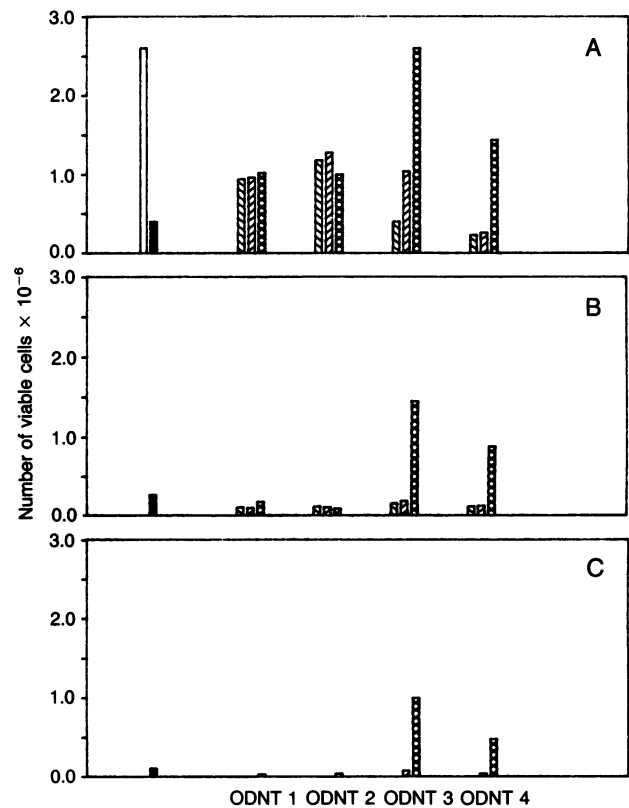


FIG. 3. Effect of oligonucleotides (ODNT) 1, 2, 3, and 4 on growth of HIV-1-infected MOLT-3 cells. The concentration of oligonucleotides 1 and 2 were 6.25 $\mu\text{g}/\text{ml}$ (\boxtimes), 25 $\mu\text{g}/\text{ml}$ (\boxplus), and 100 $\mu\text{g}/\text{ml}$ (\boxminus). The concentrations of oligonucleotides 3 and 4 were 0.1 $\mu\text{g}/\text{ml}$ (\boxdot), 1 $\mu\text{g}/\text{ml}$ (\boxtimes), and 10 $\mu\text{g}/\text{ml}$ (\boxplus). Oligonucleotides were added to infected cells at zero time (A), 24 hr after infection (B), and 48 hr after infection (C). \square , Number of viable cells in controls, which were not exposed to virus or oligonucleotides; \blacksquare , number of viable cells when the same control cells were exposed to HIV-1 (HTLV-III_B). When oligonucleotides 1 and 2 were added to HIV-1-exposed cells at zero time (A), there was an increase in viable cell numbers, but it was not dose dependent. The same concentration of oligonucleotides 1 and 2 showed no effect on growth of HIV-1-infected cells when added 24 (B) or 48 (C) hr after infection. Oligonucleotide 3 showed a dose-dependent effect and was effective even 24 or 48 hr after infection.

Anti-HIV Activity of Oligonucleotides 3 and 4 (Phosphorothioate Oligomers). Oligonucleotide 3 (antisense oligomer) showed inhibitory activity similar to that published before (11), when added at zero time. HIV expression was inhibited 80–100% at a dose of 10 $\mu\text{g}/\text{ml}$ ($\approx 1 \mu\text{M}$), and inhibition was dose-dependent (Fig. 2 and Table 1). Oligonucleotide 4, which is the mismatched sequence, showed inhibitory activity similar to that of oligonucleotide 3, when added to cell culture at zero time of infection. Oligonucleotide 3, when added 24 hr after infection, showed 80–90% inhibition of HIV expression at 1.5 μM , and, in striking contrast, *no activity* was detected by the mismatched sequence, oligonucleotide 4. After 48 hr of infection, oligonucleotide 3 gave 30–50% inhibition at similar doses and oligonucleotide 4 was inactive. Fig. 4 represents the percent inhibition of p17 protein formation in the presence of oligonucleotide 3 and oligonucleotide 4, when added 0, 24, and 48 hr after infection.

Oligonucleotides 3 and 4 both showed inhibition of HIV expression when tested against the chronically infected/uninfected cell mixture (Table 2). Oligonucleotide 3 was somewhat more active at all doses, and up to 75% inhibition was obtained at 10 $\mu\text{g}/\text{ml}$ (1.5 μM).

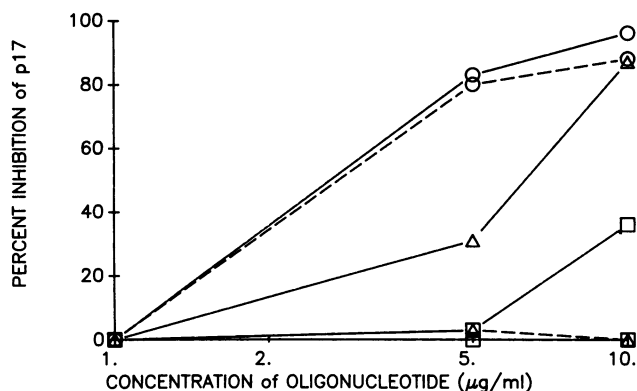


Fig. 4. Comparative inhibition of HIV-1 expression as assayed by p17 protein expression in the presence of oligonucleotides 3 (—) and 4 (---), when they were added to infected cells simultaneously with virus (○), 24 hr after infection (△), or 48 hr after infection (□). Oligonucleotides 3 and 4 showed similar activity when added at zero time of infection; oligonucleotide 4 showed no activity when added 24 or 48 hr after infection. Oligonucleotide 3 showed dose-dependent activity even when added 24 or 48 hr after infection.

Effect of Oligonucleotides 3 and 4 on Growth of HIV-1-Infected Cells. Both oligonucleotides 3 and 4 allowed improvement in growth of HIV-1-infected MOLT-3 cells, if they were added at 0 hr of infection (Fig. 3). Oligonucleotide 3 showed a dose-dependent effect and was more active than oligonucleotide 4. Even when added after 24 and 48 hr of infection, oligonucleotide 3 permitted normal cell growth.

Anti-HIV Activity of Phosphorothioate Homooligonucleotides. In a previous study (11) we found that phosphorothioate analogues of homooligonucleotides were active to approximately the same extent as the complementary and mismatched (random) phosphorothioate sequences, when they were added to cell cultures simultaneously with virus. Table 3 shows data where four phosphorothioate sequences—one complementary to the splice donor site 280–299 of HIV RNA (20), one mismatched, a dT₂₀ homooligomer, and a dA₂₀ homooligomer—were added to the cell culture at 0, 4, 8, 24, and 48 hr after infection. ID₅₀ was determined by the syncytial cell inhibition test. The ratio of the dose of oligonucleotide (in µg/ml) for ID₅₀ for 0 to 48 hr is 1:3 for oligonucleotide 5 (complementary), 1:8 for oligonucleotide 6 (mismatched), 1:9 for dT₂₀, and 1:11 for dA₂₀. Thus, the antisense oligonucleotide is clearly superior in its inhibitory potency to these other incompletely complementary oligomers, as the time after infection increases.

Toxicity of Oligodeoxynucleotides in Rats. Preliminary toxicity studies in rats were performed with oligonucleotides 1 and 3 at concentrations of 25, 50, 100, and 150 mg per kg of body weight. The dose of oligonucleotide was dissolved in 1 ml of saline and injected into 100-g rats. Each dosage level

was injected intraperitoneally into two male and two female rats. Control rats were injected intraperitoneally with 1 ml of sterile physiological saline. Neither oligonucleotide 1 nor oligonucleotide 3 showed toxicity to young males and females at the four concentrations tested. There was no evidence of lethargy or malaise in the animals at any time. The rats remained active and alert and free of visible symptoms of toxicity. None of the rats exhibited observable delayed toxicity, and no hyperirritability was noted. The body weight gain appeared to reflect a normal growth pattern.

DISCUSSION

Two types of cellular infection are described in this communication. In one case, the susceptible cells were infected 1 or 2 days prior to addition of oligomer. In this situation, every cell has been exposed to virus for 1 or 2 days prior to chemotherapy. In the other case, one part of chronically infected, virus-producing cells was added to four parts of uninfected cells. Here, the majority of cells was being exposed to fresh viral infection at the moment of addition of oligomer. In a sense, the situation is part way between “simultaneous” infection with virus and oligomer and addition of oligomer to cells that had all had previous opportunity for viral infection. The results show superior inhibitory potency of the antisense oligomers over the mismatched oligomers and homooligomers when all the cells have been previously exposed to infection (i.e., for 24 or 48 hr prior to addition of oligomer) (Table 1). This situation is somewhat comparable to that in humans already infected with HIV, whereas simultaneous infection and addition of oligomers is not.

In the simultaneously infected cells, an oligomer and the virus may have more sites of interaction than in the previously infected cells [e.g., (i) activation or inhibition of RNase H; (ii) targeting of modified oligomers, with particular attention to the oligomer receptor site of the cell membrane; (iii) influence of oligomer modifications of cell permeability and intracellular distribution; (iv) reactivity of modified oligomers with proteins, including inhibition of enzymes; (v) changes in intracellular half-lives of oligomers; and (vi) variation in toxicity to cells]. In the 24-hr-infected and chronically infected cells, a higher concentration of antisense oligomer is needed to induce the same degree of viral inhibition as in the cells in which oligomer and virus are added at approximately the same time. Where a small percentage (20%) of chronically infected cells was mixed with a large percentage (80%) of uninfected cells, the mismatched and homooligomers were more active than in the population of cells that had all been exposed to virus before addition of the oligomers. This design is more in keeping with the situation in freshly infected cells than with chronically infected cells.

These results are complementary to and in general agreement with those recently published by Matsukura *et al.* (21).

Table 3. ID₅₀ of various phosphorothioate oligonucleotides when added simultaneously with HIV and at different times after HIV infection

No.	Phosphorothioate Sequence	HIV binding site	ID ₅₀ , µg/ml				
			0 hr	4 hr	8 hr	24 hr	48 hr
5	GCGTACTCACCAGTCGCCGC	280–299	4.2	4.4	5.5	5.7 (1)	12 (3)
6	CGAGATAATGTTACACAAC	?	6	10	8.6	42 (7)	46 (8)
7	dT ₂₀	?	1.7	4	5.8	8.8 (5)	16 (9)
8	dA ₂₀	?	5.4	14.4	29	53 (10)	60 (11)

The indicated oligonucleotides were added to cell cultures 0, 4, 8, 24, and 48 hr after HIV infection. The ID₅₀ was taken as the oligonucleotide concentration (in µg/ml) that resulted in a 50% inhibition of syncytial formation. ?, See the legend to Table 1 and the Discussion; in the case of dT₂₀, there is also a poly(A) tail to be considered as a hybridization site. The numbers in parentheses are the ratios of the ID₅₀ determined when the nucleotide was added 24 or 48 hr after HIV infection to the ID₅₀ determined when the oligonucleotide was added simultaneously with HIV.

The present work presents, in addition, a comparison of the efficacy of these three above-mentioned types of oligomers in cells infected for as short a time as 4–24 hr prior to addition of oligomer. Within this time frame, the antisense oligomer is found to be much superior to the mismatched oligomer. The unmodified oligomer sequence 2 and the phosphorothioate oligomer 6 were analyzed with a computer to determine to what extent complementary segments of these sequences greater than 4 units in length occurred in the HIV genome. We chose a length of 4 contiguous bases to initiate this comparison search, since at least 4 hybridizing bases in double-stranded configuration are needed to activate RNase H, according to published reports. Seven or 8 such complementary bases are more optimal. In one of these 20-mer sequences (oligonucleotide 2) that had been synthesized for complementarity in another unrelated project and was initially considered a random control here, there were 6 bases complementary to one area in bases 917–928 and 12 bases with two mismatches complementary to the same area (917–928) that comprises the HIV genome. Similarly oligonucleotide 6 has 6 bases complementary and 17 bases with four mismatches complementary to one area in the 8421–8437 position in the HIV genome. These sequences and those chosen by other investigators (21) are therefore far from random sequence controls. The same reasoning applies to the homooligomers. The sequence TTTT occurs 49 times in the HIV genome, and TTTTT occurs 13 times. Similarly, AAAA occurs 149 times, and AAAAA occurs 50 times. The presence of complementary sequence stretches offers a logical explanation for the inhibitory activity of random controls and homooligomers in experiments from this and other laboratories. Activation of RNase H as well as hybridization arrest may likewise be involved, since as little as 4 complementary bases may activate RNase H.

In theory there is one chance in four of a particular base occurring at random at a single position in the HIV genome. The chance of a sequence of 5 bases occurring at random is therefore 4^5 or 1 in 1024. Since there are approximately 9200–9700 bases in the HIV genome, a given sequence of 5 bases may, on a random basis, occur nine times. Actually a computer search reveals a frequency of occurrence in the HIV genome of all possible tetramers ranging from 0 (CGCG) to 149 (AAAA). The range of occurrence of all possible pentamers runs from 0 (69 such) to 54 (AAGAA). A corollary to the above considerations is that although 16–20 bases in an antisense oligomer give assurance of uniqueness on a random basis, neither the human genome nor HIV represents *randomness* of sequence. It is clear that in the future more careful computer selection of random controls must be made.

How is it that our present mismatched controls and homooligomers are less effective inhibitors where susceptible cells have already been infected with HIV? A plausible explanation is that at this point the initial events of a fresh viral infection are no longer of critical importance to the replication of the virus. Once the replication cycle from proviral DNA to mRNA and viral protein synthesis is established, reverse transcription and RNase H activity related to cell entry of fresh virus are no longer as essential.

In the case of activation of latent HIV infection, the specific antisense oligodeoxynucleotides would appear to provide a more favorable therapeutic potentiality than the mismatched oligomers or homooligomers.

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