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Induction of Chronic Human Immunodeficiency Virus Infection Is Blocked In Vitro by a Methylphosphonate Oligodeoxynucleoside Targeted to a U3 Enhancer Element

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Oligodeoxynucleosides with internucleoside methylphosphonate linkages complementary to regions within U3 of human immunodeficiency virus type 1 were evaluated for their ability to block phorbol myristate acetate upregulation of virus in chronically infected promonocytic and T-lymphoblastoid cell lines. One such oligomer, targeted to an NF-κB enhancer element, inhibited phorbol myristate acetate induction of viral replication and *tat*-mediated *trans* activation of the human immunodeficiency virus long terminal repeat. The effect of this construct is contrasted with classical antisense methylphosphonate-derivatized oligomers complementary to initiation codon and splice acceptor sites of human immunodeficiency virus structural and regulatory genes. Its activity suggests a novel application of the modified oligonucleotide strategy in the blockade of viral induction from latently infected cells.

Oligodeoxynucleotides complementary to structural and regulatory transcripts of human immunodeficiency virus (HIV) can inhibit replication of this retrovirus in vitro. Initial methods for altering RNA expression were hindered by the nuclease sensitivity and low cellular uptake of these polyanions (10, 32). Such limitations have been obviated by modifications of the phosphonate backbone through the use of methylphosphonate (5, 11, 32, 38), phosphorothioate (2, 21, 25, 26), phosphoramidate (1), and phosphoroselenoate (27) linkages. Chemical alteration of the deoxyribose moiety is also being pursued (29). HIV infectivity can be blocked by such derivatized molecules targeted to initiation codon and splice acceptor (S/A) sites for HIV structural (*gag-pol*, *env*) and regulatory (*tat*, *rev*, *vif*, *nef*) transcription units as well as the viral primer-binding site for $tRNA^{Lys}$ and the 5′ untranslated genomic repeat. However, many of these oligomers concurrently inhibit normal cellular DNA synthetic responses. Consensus regions among viral and cellular S/A sites and induction of RNase H activity are potential explanations for this toxicity.

We examined viral sites distinct from these genes and contrasted them with HIV structural and regulatory units as potential targets for derivatized sequence-specific oligomers. Recognizing the importance of latent, low-level viral infection in the life cycle of HIV (12), we concentrated on a model for rescue of virus from immortalized T lymphocytes and promonocytic cells chronically infected with HIV type 1 (HIV-1). Treatment of these cells with phorbol myristate acetate (PMA) leads to viral induction as a consequence of protein kinase C activation (7, 16). An HIV enhancer sequence shared in common with NF-κB, a regulator of kappa light-chain immunoglobulin gene transcription, is one *cis*-acting element that is responsive to nuclear *trans*-acting factors activated by PMA (9). We have targeted this region, represented as a repeated element in U3 of viral RNA and in the proviral long terminal repeat (LTR), with sequence-specific methylphosphonate oligodeoxynucleosides (MP-oligomers). We demonstrate that such constructs could interfere with induction of HIV and *tat*-mediated *trans* activation with minimal cellular toxicity in a novel application of the modified oligonucleotide strategy for interference with the HIV life cycle.

MATERIALS AND METHODS

Solid-phase synthesis of oligodeoxyribonucleoside methylphosphonates. Oligomers of the type d-Np(N*p*)$_n$N, where *n* is 7 through 13, were prepared on a 1% cross-linked polystyrene support with 5′-(dimethoxytrityl)nucleoside 3′-(methylphosphonic imidazolide) intermediates (American Bionetics, Inc., Foster City, Calif.) as previously described (18).

Oligonucleoside sequences. Three different types of viral target were chosen for MP-oligomer design (Table 1). Type I included anti-initiation codon oligomers: a group antigen-polymerase polyprotein precursor transcription unit (*gag-pol*), *tat*, and an internal AUG in *pol* (initiator control). Type II included anti-S/A sites of *tat* (S/A-1 and S/A-2). Type III included elements in U3 and other regions of the HIV LTR, including a nonloop sequence in the RNA target region for Tat protein (TAR), the negative regulatory element (NRE), and the NF-κB repeated element (EN-1).

Cells. U1.1A cells were subcloned from U1, a clone of promonocytic U937 cells infected with the lymphadenopathy-associated virus strain of HIV-1 and obtained from T. M. Folks of the National Institutes of Health (7). They contain, on average, two proviral copies of HIV-1 (13). 8E5 is a $CD4^-$ cell line derived from lymphadenopathy-associated virus-infected A3.01 $CD4^+$ T lymphoblasts and was obtained from T. M. Folks (6, 8). These cells contain defective provirus and exhibit minimal expression of *trans*-acting elements in the absence of exogenous stimulation (8). All cell lines were cultured at a density of 0.5×10^6 cells per ml in RPMI 1640

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TABLE 1. Sequence specificity of MP-oligomers

MP-oligomer designation	Function of target sequence	MP-oligomer sequence	HIV target sequence[a] (nucleotide nos.)
Control globin	β-Globin	CpATTCTGT	None[b]
Control *pol*	*pol* initiator	CpATTTTTGGTTTCAA	1956 to 1969
gag-pol	*gag-pol* initiator	TpCTCTCTCCTC	324 to 333
tat	*tat* initiator	TpTTCTTGCTC	5402 to 5410
S/A-1/a	First splice acceptor site of *tat*, sequence a	CpCCAATTCTGAA	5353 to 5363
S/A-1/b	First splice acceptor site of *tat*, sequence b	ApCCCAATTCTGA	5354 to 5364
S/A-2	Second splice acceptor site of *tat*	TpGGGAGGT	7960 to 7967
EN-1	NF-κB enhancer element	TpAAAGTCCCCAG	−93 to −83
EN-1M	Mutated NF-κB	TpAAAGCTCCCAG	
NRE	Negative regulatory element	TpGTGATGAAATG	−174 to −164
TAR	TAT protein target region (sequence outside hairpin loop)	GpATCTGGTCTAA	13 to 23

[a] Numbering of HIV RNA is adopted from the proviral DNA sequence of Ratner et al. (31).
[b] Noncomplementary control.

medium (Flow Laboratories, McLean, Va.) containing 10% fetal bovine serum.

Antigen capture assay. HIV antigens were quantitated in supernatants by an enzyme-linked immunosorbent assay for viral p24 *gag* protein (Abbott Labs, Chicago, Ill.), as previously described (13).

HIV-associated *trans* activation. The ability of the *tat* transcription unit of HIV to enhance the expression of the chloramphenicol acetyltransferase (CAT) gene, when CAT is linked to the LTR of HIV, was measured by standard techniques detailed elsewhere (13, 14). They involved DEAE-dextran-mediated cotransfections and thin-layer chromatographic analyses of [^{14}C]chloramphenicol-labeled cellular extracts. The *tat* plasmid pIIIextat contains pBR322 regulatory sequences, two exons of the HIV-1 *trans* activator *tat*, and the 5′ LTR of HIV-1 (strain HTLV-IIIB), representing nucleotides −167 to +80. The *tat* plasmid pCV-1 contains a 1.8-kb fragment of HIV-1 cDNA encompassing *tat*, without S/A sequences. In samples treated with MP-oligomers, cells were preincubated for 1 h before transfection with the appropriate concentration of oligodeoxynucleoside, washed, then exposed to plasmid DNAs. After DNA transfer, cells were washed and incubated in 0.5 ml of culture medium, again containing oligomer, for 48 h.

HIV infection. Peripheral blood mononuclear cells (PBMC) obtained from HIV-seronegative donors were activated with 2 μg of phytohemagglutinin (PHA) per ml for 24 to 72 h, washed with phosphate-buffered saline, and then suspended at 2×10^6 cells per ml in culture medium (RPMI 1640 containing 10% fetal bovine serum and 64 U of nonrecombinant interleukin-2 [ElectroNucleonics, Inc., Silver Spring, Md.] per ml) in polyvinyl flat-bottom macrowells. Then 1,000 50% tissue culture infectious doses of viral stock, representing supernatants of H9 T lymphoblasts infected with the HTLV-IIIB strain of HIV-1 (14), were added, and the medium was completely changed 18 h later. One half of the medium was removed and replaced with fresh culture medium every 3 or 4 days. Infection was followed by particulate reverse transcriptase assay of culture supernatants (15) or by enzyme-linked immunosorbent assay for p24 core antigen.

RESULTS

HIV *trans* activation and *tat* antisense strategies. Several studies have suggested that *tat* is a potent target for sequence-specific oligomers when utilized in an acute in vitro infection system (1, 38). We evaluated the activity and specificity of an anti-S/A *tat* oligomer in cells chronically infected with HIV. In the U1.1A promonocytic line bearing two proviral copies per cell, <2% basal conversion of [^{14}C]chloramphenicol into its acetylated forms was noted by transfection with the HIV-LTR-CAT plasmid alone (Table 2). In contrast, introduction of exogenous *tat* by cotransfection of these cells with HIV-LTR-CAT and either *tat* plasmid led to detection of three acetylated forms of chloramphenicol and enhancement of CAT activity by 5- to 19-fold (Table 2).

The issue of specificity was addressed by contrasting oligomer effects in cotransfections with plasmids containing a single coding region of *tat* (pCV-1) or including the *tat* S/A-1 sequence (pIIIextat). The addition of 25 to 100 μM anti-S/A-1 oligomer had little effect on CAT activity in the presence of pCV-1 (Table 2). In contrast, an identical system utilizing pIIIextat was affected by S/A-1. The range of inhibition varied from a low of 32% to a maximum of 76% at 100 μM (Table 2) with a mean of 60% in five separate experiments. Similar inhibition was obtained with a related oligomer, S/A-2 (50 to 99% at 100 μM; data not shown). The anti-*tat* initiation codon oligomer had no effect (<10% inhibition; data not shown).

HIV infectivity and anti-initiation versus anti-S/A oligomers. The effect of these constructs on HIV infectivity of

TABLE 2. Effect of anti-*tat* splice acceptor site MP-oligomers on *tat*-mediated *trans* activation in U1.1A cells[a]

Plasmid transfected		Total MP-oligomer concn (μM)[b]	Exp 1		Exp II	
HIV-LTR-CAT	*tat*		CAT activity[c]	% Inhibition	CAT activity	% Inhibition
+			1.6		0.9	
+	pCV-1		31.1		4.6	
+	pCV-1	100	31.9	0	4.0	16.2
+	pIIIextat		14.5		6.8	
+	pIIIextat	25	23.8	0	6.4	6.8
+	pIIIextat	50	10.3	32.6	4.7	35.6
+	pIIIextat	100	4.7	76.0	4.9	32.2

[a] U1.1A cells (2×10^6 per condition) were exposed to 1 μg of DNA from each plasmid for cotransfections or salmon sperm DNA for controls (*tat*-negative cultures). Oligomers were present at the start of transfection and throughout the 48-h culture period.
[b] Equimolar amounts of S/A-1a and S/A-1b were used.
[c] Percent acetylation of [^{14}C]chloramphenicol.

TABLE 3. Effect of MP-oligomers on HIV-1 infection of activated PMBCs[a]

HIV	MP-oligomer (μM)	Reverse transcriptase[b]	
		Mean cpm ± SD	% Inhibition
Expt A			
−		2,755 ± 205	
+		70,293 ± 16,101	
+	Control *pol* (50)	69,575 ± 11,595	1.1
+	*tat*-AUG (50)	72,504	0
+	Anti-S/A-1a (50)	8,674	91.2
Expt B			
−		2,405 ± 1,100	
+		21,727 ± 170	
+	Anti-S/A-1a (5)	20,418 ± 5,125	6.8
+	Anti-S/A-1a (10)	24,927 ± 2,370	0
+	Anti-S/A-1a (20)	19,895 ± 9,295	9.5
+	Anti-S/A-1a (25)	18,745 ± 1,345	15.4
+	Anti-S/A-1a (50)	12,890 ± 1,841	45.7

[a] PMBC (2.75×10^5) prestimulated with PHA for 2 days were incubated with buffer or oligomer for 1 h at 37°C and then exposed to 1,000 50% tissue culture infectious doses of HIV-1 for 2 h at 37°C. Cells were washed and incubated, together with fresh oligomer, in flat-bottom microwells in total volume of 0.3 ml.

[b] Supernatants were removed 7 days after HIV exposure. Data represent the mean incorporation of [^{3}H]dTMP into DNA for three separate cultures with a poly(rA)-oligo(dT) template. The percent inhibition was calculated as [(positive control − experimental value)/(positive control − negative control)] × 100.

PHA-stimulated PBMCs was next addressed. Initiation codon (AUG) oligomers against *tat* or *pol* had no effect on viral infection or replication, as determined by measurement of particulate reverse transcriptase activity (Table 3). In contrast, MP-oligomers directed against the S/A sites of *tat* markedly blocked HIV replication. Use of lower concentrations of overlapping nucleotides in the region of S/A-1 were less effective than a higher dose of one sequence alone at blocking *tat* activity or HIV replication (data not shown).

The possibility that targeting S/A sequences might concurrently interfere with normal cellular functions is raised by the similarity of the HIV strain HTLV-IIIB-activated S/A sequence CCATTTTCAGAATTGGGTGT (39) to the cellular consensus sequence PyNPyPyPyNCAG (28). This was investigated by examining the effects of various MP-oligomers on DNA synthetic responses in activated PBMCs and in an immortalized CD4$^+$ T-cell line, SK7. Oligomers that had no effect on HIV replication or HIV LTR associated *trans* activation did not interfere with [^{3}H]thymidine incorporation into either cell type (Table 4). EN-1 gave <15% inhibition in four separate experiments, whereas S/A-1 and −2 at 50 μM decreased incorporation by 30 to 50%. Therefore, inhibition of HIV-directed CAT transcription and particulate reverse transcriptase production by the latter could be due, in part, to cell toxicity. We thus investigated an additional set of modified oligomers in activated U1.1A cells.

Induction of HIV replication and anti-U3 oligomers. Protein kinase C-mediated activation of HIV in U1.1A cells has been suggested as a model for the latent lytic cycle of HIV replication (16). This process is synergistic with *tat* activity (16), involves NF-κB sites (9), and appears to recapitulate the molecular events of a single-cycle infection of highly susceptible cells. One semiquantitative measure of this process is PMA enhancement of LTR-CAT. Conversion of radiolabeled chloramphenicol to its acetylated forms was stimulated threefold by PMA when the HIV-CAT and *tat* plasmids were cotransfected into U1.1A cells (Fig. 1A). MP-oligomers targeted to NRE and *gag-pol* had no effect in this system, either on baseline *tat*-mediated upregulation of HIV-LTR-CAT or on PMA enhancement of this function (data not shown). Similarly, our anti-TAR oligomer had no effect. (It should be noted, however, that this latter construct was not complementary to the hairpin loop or stem-bulge regions of TAR, now known to be critical for the binding of Tat or *tat*-induced cellular proteins [4].)

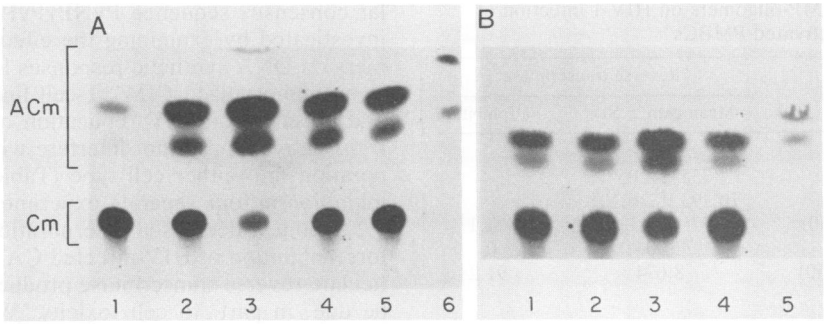
In contrast, the MP-oligomer complementary to a U3 and LTR enhancer sequence, EN-1, blocked PMA-mediated enhancement of *tat* by ≥90% (Fig. 1A), while having a reproducible but much lower effect (≤15% suppression) on baseline *tat* activity (Fig. 1A; compare lanes 2 and 5). This was true not only in these promonocytic cells but also in 8E5 cells (immortalized cells of CD4$^+$ T-lymphocyte lineage). Unlike U1.1 cells, 8E5 cells harbor *pol*-defective proviruses (8). They already express maximal stimulation of LTR-CAT, as reported previously (6) and noted by the failure of

TABLE 4. Effect of MP-oligomers on DNA synthetic responses of mitogen-stimulated and transformed cells[a]

Cell	Oligomer (μM)	Cellular proliferation, cpm (% inhibition)	
		Expt I	Expt II
PHA-PBMC		19,647 ± 1,103	37,136 ± 5,708
PHA-PBMC	*gag-pol* (50)	18,542 ± 1,618 (5.6)	33,125 (10.8)
PHA-PBMC	S/A-1a (50)	16,718 ± 897 (14.9)	ND[b]
PHA-PBMC	S/A-1a (50)	16,314 ± 208 (17.0)	24,536 (33.9)
PHA-PBMC	EN-1 (10)	ND	39,620 ± 613 (0)
PHA-PBMC	EN-1 (50)	ND	32,867 ± 499 (11.5)
SK7		1,065 ± 99	7,800 ± 100
SK7	*gag-pol (50)*	1,055 (0)	6,600 (15.4)
SK7	S/A-1a (25)	810 ± 80 (24.0)	6,700 (14.1)
SK7	S/A-1a (50)	719 ± 60 (32.5)	4,100 (47.4)
SK7	S/A-1a (100)	647 ± 7 (39.3)	ND
SK7	EN-1 (50)	1,104 ± 24 (0)	ND

[a] PBMC (2.5×10^5 per condition) were incubated with PHA (10 μg/ml) and oligomer for 3 days, during the final 18 h of which 1 μCi of [^{3}H]thymidine was added. SK7-immortalized lymphoblasts (10^5) were similarly cultured, except that PHA was not used. Cellular proliferation represents the mean counts per minute ± standard deviation of triplicate wells.

[b] ND, Not determined.



	1	2	3	4	5
Conversion (%)	2.3	35.5	96.5	42.1	29.8
Co-transinfected:					
HIV-LTR-CAT	+	+	+	+	+
tat (pIIIextat)	-	+	+	+	+
PMA (50 ng/ml)	-	-	+	+	-
EN-1 (100μM)	-	-	-	+	+

	1	2	3	4
Conversion (%)	17.8	17.1	71.6	13.6
Co-transinfected:				
HIV-LTR-CAT	+	+	+	+
tat	-	+	+	+
PMA (50 ng/ml)	-	-	+	+
EN-1 (100μM)	-	-	-	+

FIG. 1. (A) Effect of EN-1 methylphosphonate oligomer on PMA-mediated enhancement of HIV-LTR-CAT–*tat* interactions in promonocytic cells. The CAT plasmid (1 μg DNA) was cotransfected into U1.1A cells (2×10^6) with 1 μg of salmon sperm DNA (lane 1) or the pCV-1 plasmid containing *tat* (1 μg; lanes 2 through 5). PMA (50 ng/ml) and/or EN-1 (100 μM) was present throughout the 48-h culture period. Conversion of [^{14}C]chloramphenicol (Cm) to its acetylated forms (ACm) was determined. Lane 6 represents a positive control for the CAT enzyme. (B) Effect of EN-1 methylphosphonate oligomer on PMA-mediated enhancement of HIV-LTR-CAT–*tat* interactions in T cells. The CAT plasmid (1 μg of DNA) was cotransfected into 8E5 cells (2×10^6) with 1 μg of salmon sperm DNA (lane 1) or a plasmid containing *tat* (1 μg; lanes 2 through 4). PMA (50 ng/ml) and/or EN-1 (100 μM) was present throughout the 48-h culture period. Conversion of [^{14}C]chloramphenicol (Cm) to its acetylated forms (ACm) was determined. Lane 5 represents a positive control for the CAT enzyme.

exogenous *tat* to augment LTR-CAT activity (Fig. 1B, compare lanes 1 and 2). A dose-response curve (Fig. 2) revealed a 50% inhibitory concentration for EN-1 of approximately 25 μM in this system.

A parallel effect of EN-1 on induction of viral replication was observed. EN-1 blocked PMA-mediated upregulation of particulate HIV-1 from U1.1A cells in a dose-dependent manner (Table 5). The anti-NRE and -TAR oligomers again had no effect (Table 5).

The issue of specificity was further defined for EN-1 by employing a mutated construct, TpAAAGCTCCCAG, with the two central bases reversed (Table 1). In one of three representative experiments, <2% inhibition of PMA-mediated upregulation of LTR-CAT activity in concert with *tat* was observed (Fig. 3). In addition, no significant alteration of HIV induction by PMA occurred (Table 5, experiment C).

DISCUSSION

Certain regions of HIV genomic RNA and mRNA transcripts are particularly sensitive to targeting by modified oligonucleosides. In many instances, however, concentrations of oligomer that are effective at blocking HIV replication concurrently depress cell growth (1, 10). This has led some investigators to postulate that certain constructs, particularly unmodified homo-oligonucleotides, may inhibit HIV by mechanisms other than antisense competitive hybridization (1).

We have demonstrated similar effectiveness and nonspecific toxicity of our *tat* anti-S/A oligomers. In contrast, one construct targeted to the U3 region of HIV RNA and complementary to the NF-κB enhancer element in the HIV LTR had much less of an effect on cellular DNA synthetic responses but blocked the upregulation of virus upon exposure to a protein kinase C activator, PMA. This effect was specific, since a mutated construct had no effect. Both virion production and HIV LTR-directed transcriptional regulation were altered.

EN-1 also had a consistent but minor effect on *tat* function in the absence of PMA. This is consistent with the finding that *tat* can function independently of PMA-mediated effects involving NF-κB (35) and possibly other transcription factors (33). However, since there is some synergism between PMA induction and *tat* activity (16), some inhibitory effect was anticipated.

The long latency period for development of clinical AIDS is thought to be influenced by factors that activate HIV replication in different cell types. In vitro, activation of the HIV-LTR is associated with increased protein binding to LTR sequences in a region including the NF-κB core enhancer, represented as GGGACTTTCC (nucleotides −104 to −95) and GGGACTTTCC (nucleotides −90 to −81) of HIV-1, and a single sequence in HIV-2. In this study PMA was utilized as a potent and general viral inducer. However, the phorbol ester effect may be generalized to other mechanisms of induction of latent HIV. Signals as disparate as herpesvirus coinfections (3, 9, 12) and cytokine exposure or antigenic stimulation (reviewed in reference 12) involve protein kinase C activation and/or induction of nuclear factors that bind to or near NF-κB sequences. Earlier efforts with modified oligomers have shown variable effects on constitutive expression of virus by chronically infected T-cell lines (26, 27), and chronically infected cells of the monocyte-macrophage lineage had not previously been examined. This is clearly an important area, since classic antiviral agents such as 3′-azido-3′-deoxythymidine are in-

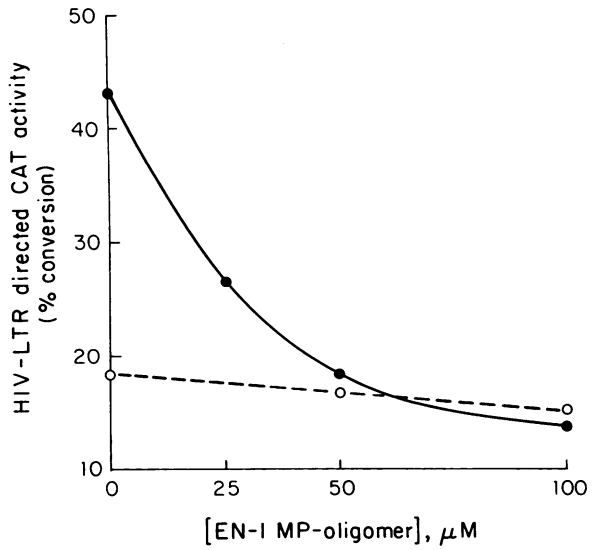


FIG. 2. Dose-response curves for the effect of EN-1 oligomer on PMA-mediated enhancement of HIV-LTR-CAT. The CAT plasmid (1 μg of DNA) was cotransfected into 8E5 T-lymphoblastoid cells with a plasmid containing *tat* (1 μg of DNA). PMA (50 ng/ml) and/or oligomer was present throughout the 48-h culture period. Conversion of [^{14}C]chloramphenicol (Cm) to its acetylated forms (ACm) was determined. Cells were cultured in the presence (●) or absence (○) of PMA.

capable of suppressing activation of HIV in chronically infected promonocytic or T-cell lines (30).

Impediments to this antisense strategy often focus on issues of specificity. There are approximately 3×10^9 nucleotides in the human genomes (39); the sequence of the 12-mers used in our experiments would be expected to occur at random about 1 time in 10^7. However, altering two bases in EN-1 was sufficient to abrogate its activity. The rapid alteration in HIV sequences, attributed in part to the high

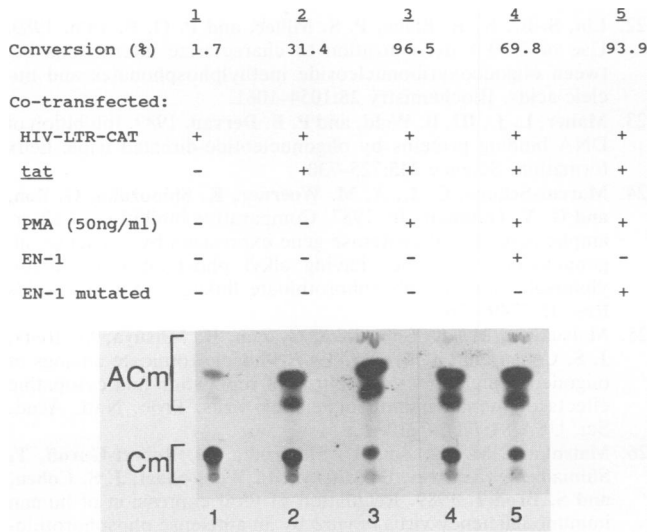


FIG. 3. Specificity of EN-1 methylphosphonate oligomer on PMA-mediated enhancement of HIV-LTR-CAT-*tat* interactions in U1.1A cells. Cotransfections were carried out as described in the legend to Fig. 2, except that U1.1A cells were used.

TABLE 5. Effect of MP-oligomers on phorbol ester-mediated upregulation of HIV replication in chronically infected U1.1A cells[a]

PMA	MP-oligomer		HIV-1 activity[b] (% inhibition)
	Specificity	Concn (μM)	
Expt A			
−			3,235 ± 90
+			12,260 ± 4,870
+	EN-1	10	6,810 ± 5 (60.4)
+	EN-1	50	5,090 ± 220 (79.4)
+	EN-1	100	4,140 ± 1,070 (90.0)
+	NRE	100	9,650 ± 90 (33.4)
Expt B			
−			2,560 ± 320
+			53,700
+	EN-1	10	68,900 (0)
+	EN-1	100	35,400 (35.8)
+	S/A-1b	10	133,400 (0)
+	S/A-1b	100	79,900 (0)
Expt C			
−			1,560 ± 250
−	EN-1	50	1,490 ± 81 (4.5)
+			5,720 ± 1,600
+	EN-1	5	5,090 ± 1,070 (15.1)
+	EN-1	50	4,230 ± 1,490 (35.8)
+	EN-1M	50	5,985 (0)
+	S/A-1a	50	7,680 ± 610 (0)
+	TAR	100	6,450 ± 190 (0)

[a] Cells were plated at 10^4 per microwell in 0.2 ml of culture medium together with 5 ng of PMA per ml. Supernatants were harvested 48 h later and tested for HIV-1 p24 core antigen by an enzyme-linked immunosorbent assay-based antigen capture system. Each p24 value represents the mean of two assays on pooled samples of triplicate wells. Percent inhibition was calculated as described in footnote *b* of Table 3.

[b] Picograms of p24 antigen per 10^4 cells.

error rate of its polymerase (34), does introduce another variable. Still, the *tat* S/A site is maintained among all known HIV sequences (39). Similarly, the NF-κB enhancer is conserved among isolates of HIV-1 and HIV-2.

The possibility of viral or cellular proteins binding to the viral RNA or DNA and preventing access to MP-oligomers must also be considered. This is particularly relevant with regard to targeting of U3 regions, which are duplicated in enhancer elements of the proviral DNA. DNase I footprinting has shown that this region is open and available for binding in the uninduced state, in promonocytic cells as well as cells of other lineages, and that protein binding occurs only after protein kinase C activation (37).

The mechanism(s) of action of the EN-1 oligomer remains speculative, however. We hypothesize that EN-1 may have a dual role, inhibiting reverse transcription by blocking the U3 region in the genomic RNA as well binding to the enhancer element in the proviral LTR. Experiments are in progress to assess the latter possibility; double-stranded DNA can serve as a target for formation of a triple helix (23), particularly in the presence of polypurine tracts. A 16-mer polypurine sequence (AAAAGAAAAGGGGGGA; nucleotides −470 to −455) is immediately adjacent to U3 in HIV-1 (31).

In terms of clinical utility, oligomers such as EN-1 would be unlikely to have an effect on active viral replication and spread, since NFκB enhancer elements are not essential for

HIV replication (4). Constructs of this type might be most useful in latent phases of infection or when employed with antisense oligonucleotides directed against classic viral structural and regulatory targets. In addition, unless significant sequestration of a compound can be demonstrated, the concentrations of modified oligomers required must be much lower. Further derivatization of these constructs may enhance their potency and decrease the effective dose, bringing them into the range of potential therapeutic application. For example, directed cleavage of polynucleotides has been obtained by covalent linkages of oligomers to iron porphyrins (17), intercalating agents (36), and EDTA (22). Psoralen conjugation can decrease the concentrations required for inhibition of translation by 1 or 2 log units (18). Cellular targeting may also be refined as antibody coating of liposomes bearing such constructs is explored (20).

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