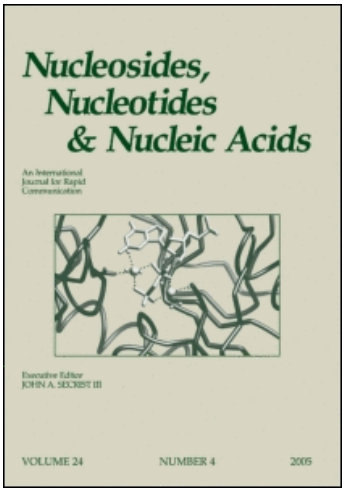

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ANTISENSE OLIGODEOXYNUCLEOTIDE COMPLEMENTARY TO CXCR4 mRNA BLOCK REPLICATION OF HIV-1 IN COS CELLS

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ABSTRACT: CXCR4 is both a chemokine receptor and an entry co-receptor for the T-cell line-adapted human immunodeficiency virus type 1 (HIV-1). To find a more efficacious therapeutic treatment of acquired immunodeficiency syndrome, we examined the effects of antisense oligonucleotides on CXCR4 production. COS cells , stably expressing CXCR4 and CD4, were incubated with several kinds of oligonucleotides. Total human p24 antigen production was determined using an enzyme-linked immunosorbent assay system. An antisense phosphorothioate-modified oligonucleotide, complementary to the translation initiation region of the CXCR4 mRNA, showed minimal inhibition of p24 antigen production at the high concentration of 2μM. On the other hand, the antisense phosphorothioate oligonucleotide, when used with transfection reagents, showed high efficiency at low concentrations, and confirmed the sequence-specific action. Interestingly, the oligonucleotide with the natural phosphodiester backbone, when used with the transfection reagents, also had high functional effects, comparable to the modified oligonucleotide. This study defines the prerequisite criteria necessary for the design and the application of antisense oligonucleotides against HIV-1 *in vivo*.

INTRODUCTION

Human immunodeficiency virus type-1 (HIV-1) entry into cells requires interactions with certain co-receptors in addition to CD4. Several studies have shown that the β-chemokine receptor CCR5 acts as the major receptor for primary non-T-cell-line-adapted viruses, i.e., slow/low, NSI isolates. Primary isolates with the rapid/high, SI phenotype and T-cell-line-adapted HIV-1 strains instead use the α-chemokine

receptor CXCR4 (previously called fusin or Lestr). The suppression of the activities of HIV-1 co-receptors might provide a good therapeutic strategy for some HIV diseases; however, the results have not always been satisfactory.

On the other hand, the use of antisense oligonucleotides has been regarded as a potential therapeutic approach with reference to the gene levels. In the present study, we have used antisense oligonucleotides to try to modulate the production of the HIV-1 second receptor, CXCR4, in COS cells stably expressing CD4 and CXCR4.

Phosphorothioate oligonucleotides and phosphodiester oligonucleotides (S- and D-oligomers, respectively) were synthesized automatically (FIG.1).

ANS : 5' - CAT GGT AAC CGC TGG TTC TC - 3'
SEN : 5' - GAG AAC CAG CGG TTA CCA TG - 3'

FIG.1. Sequences of oligonucleotides tested for the inhibition of CXCR4 production. The 20 mer antisense sequence is complementary, bases to (-60) to -79 of the human CXCR4 mRNA sequence. Abbreviations: ANS, antisense-sequence; SEN, sense-sequence.

RESULTS AND DISCUSSION

The inhibitory effects of the antisense S-oligomer on CXCR4 production by COS cells were examined. The COS cells were grown in DMEM with 10% fetal calf serum, 500μg/ml G418, and 200μg/ml hygromycin B. The T-cell -tropic HIV-1 strain NL4-3 virus (19ng/ml) was added to the COS cells seeded at 1×10^5 cells/well. After 24 hr of infection, the cells were gently washed three times with fresh media to remove the viral inocula, and then various concentrations of oligomers were added to each well. After 2 days, the soluble HIV-1 p24 concentrations in the supernatants were detected using the respective enzyme-linked immunosorbent assay system (ELISA). The NL4-3 virus infection was suitable for HIV-1 replication, because the total p24 concentration in the culture supernatant was sufficient for the ELISA detection (50pg/ml). The viability was more than 95% as assessed by the trypan blue exclusion method, at a 2μM concentration of D- and S-oligomers.

We selected an antisense sequence, including the initiation codon. Since it has been suggested as a target site in several other mRNAs, many investigators have chosen to target the translation initiation site. We observed the inhibitory effects of various concentrations of S-oligomers on p24 antigen production in the medium. Nether the antisense nor the sense D-oligomers reduced CXCR4 production at all, even at the highest concentration (2μM) and a 48 hr sufficient incubation time (data not shown). In

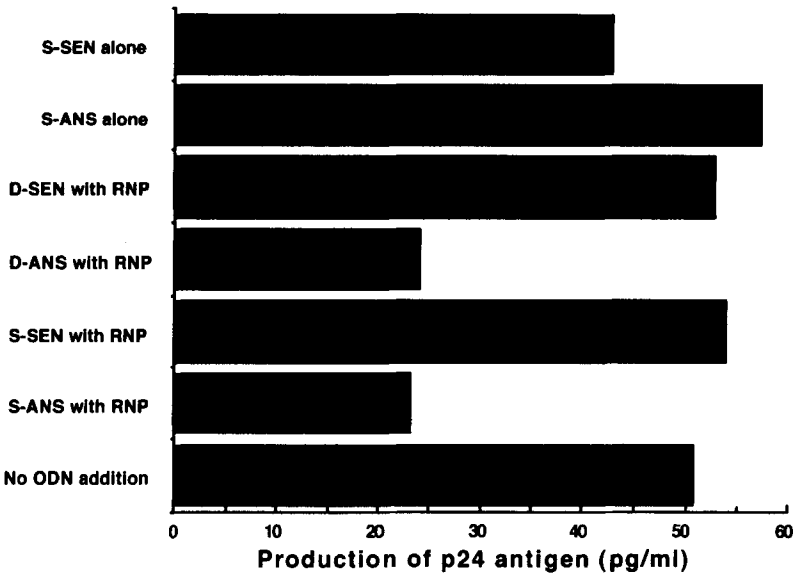

FIG.2. Inhibition of p24 antigen production by antisense D- and S-CXCR4 oligonucleotides to COS cells. The concentration of each oligonucleotide was 2μM. The experiments with the peptide transfection reagent showed significant changes in the detection of p24 antigen.

contrast, the S-oligomers showed a slight antisense effect at their high concentration; as compared with the control without the oligomer (data not shown).

The naturally occurring phosphodiester backbones of oligonucleotides are highly sensitive to enzymatic degradation; therefore, they are not suitable for use in antisense therapy. Synthetic oligonucleotides, with altered chemistries, have been designed to bypass this problem. One such modification , the phosphorothioate backbone, is made by replacing one non-bridging oxygen of each internucleoside phosphate in the phosphodiester with sulfur. This substitution substantially decreases the degradation of the oligonucleotides by nucleases, thereby making them better candidates for *in vivo* use. However, the preparation of such modified oligomers is difficult, due to problems in isomer separation and the lack of specificity of the target RNA sequences. A better solution is to utilize molecular assembly, without resorting to the chemical modification of oligomers. Moreover, to be useful as antisense effectors, the oligomers must be delivered into the cytosol, the nucleus, or both to find their target RNA or DNA.

Next, we examined the effects of transfection reagents on the antisense activity (FIG.2). The antisense S-oligomers encapsulated in the original peptide transfection

reagent (RNP) showed more functional effects. At the lowest concentration, the antisense effect on p24 production of COS cells was almost two-fold enhanced as compared with that of the S-oligomer itself. Interestingly, the non-modified D-oligomers were as active as the modified S-oligomers, in a sequence specific manner.

Since high concentrations, equal to and more than 2μM of antisense oligomer, were necessary to inhibit the p24 production, and HIV replication, we tested a basic peptide as a cell transfection reagent. Our data suggest that the reagent enhanced the antisense activities about 2-fold. Pichon *et al.* reported that an amphiphilic and basic anionic peptide might be a good delivery system for antisense oligomers, as demonstrated in human non small cell lung carcinoma cells. However, further studies for a better delivery system of our CXCR4 antisense oligomers will be necessary.

Thus, we conclude that antisense oligomers to the second HIV-1 receptor, CXCR4, efficiently inhibit p24 production when used with the RNP transfection reagent. The antisense S- and/or D-oligomers targeted to the sequence including the initiation region of the HIV-1 second receptor, CXCR4, are a potential tool for HIV gene therapy.

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