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Selective Inhibition of Monocyte Chemoattractant Protein-1 Gene Expression in Human Embryonal Kidney Cells by Specific Triple Helix-Forming Oligonucleotides

Petra Marchand, Klaus Resch, and Heinfried H. Radeke

Monocyte chemoattractant protein-1 (MCP-1) is a chemokine that is expressed by a variety of tissue cells in response to inflammatory stimuli, such as IL-1β, TNF-α, and IFN-γ. A major function of MCP-1 is the recruitment and activation of monocytes and T lymphocytes. Overexpression of MCP-1 has been implicated in a number of diseases, including glomerulonephritis and rheumatoid arthritis, indicating that the modulation of MCP-1 activity and/or expression is a desired therapeutic strategy. In the present study, our aim was to test whether the MCP-1 expression could be inhibited at the transcriptional level using triple helix-forming oligonucleotides (TFOs). We designed a TFO targeted to the SP-1 binding site in the human MCP-1 gene promoter. Gel mobility shift assays demonstrated that the phosphorothioated oligonucleotide was also effective in this assay with an 8-fold higher EC50 value. Binding of the TFO to the target DNA prevented the binding of rSP-1 and of nuclear proteins in vitro. The TFO could also partially inhibit endogenous MCP-1 gene expression in cultured human embryonic kidney cells. Treatment of TNF-α-stimulated human embryonic kidney 293 cells with the TFO inhibited the secretion of MCP-1 in a dose-dependent manner (up to 45% at 5 μM oligonucleotide). The inhibition of MCP secretion was caused at the level of gene transcription, because MCP-1 mRNA levels in oligonucleotide-treated cells were also decreased by ~40%. The Journal of Immunology, 2000, 164: 2070–2076.

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1 Abbreviations used in this paper: MCP, monocyte chemoattractant protein; HEK, human embryonic kidney; TFO, triple helix-forming oligonucleotide.
the target DNA exist per cell, compared with hundreds or thou-
sands of RNA and protein targets for antisense oligonucleotides
taptamers. It has been demonstrated that triplex formation at a
promoter can block the binding of various transcription factors,
including SP-1, thereby inhibiting transcription initiation (re-
viewed in Ref. 19). Inhibition of endogenous gene expression by
cellular TFO treatment has also been observed; examples include
the IL-2R (20), TNF-α (21), and GM-CSF (22).

The mechanisms underlying triple helix formation are reason-
ably well understood. TFOs bind to their dsDNA target in a
sequence-specific manner. Triplex formation has only been observed
at homopurine regions of DNA (19). The TFO binds in the major
groove of DNA, forming Hoogsteen or reverse Hoogsteenhydro-
gen bonds with bases in the purine-rich strand. The TFO itself can
consist of either pyrimidines or purines. Pyrimidine-containing
TFOs generally bind parallel to the purine-rich strand; sequence
specificity is mediated by specific binding of thymine bases to A:T
base pairs and protonated cytosine bases (C+) to G:C base pairs.
Because cytosine is only protonated under acidic pH, pyrimidine
TFOs do not usually bind to duplex DNA under physiological pH
without base modifications. Purine-containing oligonucleotides
bind antiparallel to the purine-rich strand in the target DNA, and
the binding occurs readily at physiologic pH. Sequence specific-
ity is mediated by binding of G to G:C and T to A:T base pairs.

The aim of the present study was to test whether the MCP-1
gene expression can be inhibited at the level of transcription using
the TFO technology. A 19-bp TFO was designed that targets the
binding site for the transcription factor SP-1 and a consensus se-
try nucleotides to duplex DNA was confirmed by native polyacyr
amide gels electrophoresis through 12.5% gels, followed by ethidium bromide staining.

Triple helix gel shift assays

Double-stranded MCP-1 promoter DNA was 3’ end labeled with digoxi-
genin-11-dUTP and terminal deoxynucleotidyl transferase using reagents of the DIG
Gel Shift Kit (Roche Molecular Biochemicals), according to the instruc-
tions of the manufacturer. To assess triple helix formation, band shift ex-
periments were performed essentially as described by Durland et al. (25).
Briefly, 60 fmol of the labeled duplex DNA was incubated at 20°C
with the indicated concentrations of triple helix-forming or control oligo-
nucleotides in a buffer consisting of 10 mM Tris-HCl (pH 7.4), 5 mM
MgCl₂, and 10% sucrose. Immediately following the incubation, the samples
were electrophoresed through 12.5% native polyacrylamide gels buff-
ered with 89 mM Tris, 89 mM boric acid, and 5 mM MgCl₂. Electrophores-
isis was at 80 V for 2 h at 4°C. The DNA was then transferred onto nylon
membrane by electroblotting in DNA transfer buffer (22 mM Tris, 22 mM
boric acid, pH 8). The digoxigenin-labeled DNA probes and complexes
were detected on the nylon membrane using the DIG Chemiluminescent
Nucleic Acid Detection Kit (Roche Molecular Biochemicals).

Protein-binding assays

MCP-1 promoter duplex DNA was incubated with 10 μM TFOs and/or
binding proteins, as indicated. For the experiment with purified SP-1, 100
fmol of MCP-1 promoter duplex was incubated with 4 footprinting units of
human SP-1 (Promega) for 1 h at 20°C in the presence or absence of 10
μM TFO in a buffer that consisted of 10 mM Tris-HCl (pH 7.4), 10%
sucrose, 20% glycerol, 20 mM KCl, 1 mM DTT, 4 mM HEPES, and 5 mM
MgCl₂. For the experiment with nuclear extract proteins, 100 fmol of
MCP-1 promoter duplex was incubated for 15 min at 20°C with 15 μg of
nuclear extract proteins from unstimulated or TNF-α-stimulated HEK 293
cells (see below) in the presence or absence of 10 μM TFO in a buffer
consisting of 10 mM Tris-HCl (pH 7.4), 10% sucrose, 5 mM MgCl₂, and
1 μg poly(dAT)). All samples were electrophoresed through 7% poly-
acrylamide gels buffered with 89 mM Tris, 89 mM boric acid, and 5 mM
MgCl₂. The samples were transferred to nylon membrane, and digoxi-
genin-labeled DNA was detected using the DIG Chemiluminescent Nucleic
Acid Detection Kit (Roche Molecular Biochemicals).

Cell culture, oligonucleotide, and cytokine treatment

HEK 293 cells were cultured in DMEM supplemented with 10% FCS, 100
U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. For analysis
of cytokine-induced stimulation of chemokine production, the cells
were plated in 24-well plates at a density of 160,000 cells/well and grown
to 80% confluency. Then the culture media were replaced with 400 μl
of fresh medium per well, and the cells were left unstimulated or treated
with various cytokines (500 U/ml TNF-α, 10 ng/ml IL-1β, or 1000 U/ml IFN-γ)
for 24 h. For oligonucleotide treatment experiments, the cells were also
plated in 24-well plates at a density of 160,000 cells/well. On the following
day, the cells were pretreated with 2 μM or 5 μM phosphorothioated TFO
or control oligonucleotide for 48 h. The oligonucleotides were added di-
rectly to the culture medium without the addition of a transfection reagent.
After a 48-h incubation, the medium was removed, fresh medium (400
μl/well) was added containing the same concentration of oligonucleotide
as before, and the cells were cultured for additional 24 h in the absence
or presence of 500 U/ml TNF-α. At the end of each experiment, the culture
media were harvested, centrifuged for 5 min at 12,000 × g to remove cell
debris, and stored at −20°C until analysis of secreted proteins by ELISA.

For Northern analysis, HEK 293 cells were treated for 48 h with 5 μM
phosphorothioated TFO or control oligonucleotide. The cells were then
stimulated with 500 U/ml TNF-α or left unstimulated. After 3 h of stim-
ulation, the cells were harvested, and total RNA was isolated.

Preparation of nuclear extract proteins

A total of 1 × 10⁷ unstimulated or TNF-α-stimulated (500 U TNF-α/ml,
24 h) HEK 293 cells was trypsinized, washed twice with ice-cold PBS, and
resuspended in 400 μl buffer A (10 mM HEPES (pH 7.9), 10 mM KCl, 0.1
mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, and 10 μg/ml
leupeptin). The samples were mixed for 30 s and immediately centrifuged
at 13,000 rpm for 5 min at 4°C, the resulting supernatant con-
tained the nuclear extract proteins. The protein concentrations were deter-
mined using the Bradford Protein Assay (Bio-Rad, Hercules, CA) with
BSA as standard.
In vitro determination of triple helix formation by gel mobility shift assay. The TFO or control oligonucleotide to duplex DNA was assayed by native gel electrophoresis through 12.5% polyacrylamide gels. The target duplex was the digoxigenin-3′ end-labeled 39-bp fragment spanning bp −76 to −38 of the MCP-1 promoter, shown in Fig. 1. Control duplex 1 was a 39-mer digoxigenin-labeled duplex provided by the DIG Gel Shift Kit (Roche Molecular Biochemicals); the second control was a fragment containing the SP-1 binding site of the human MCP-1 promoter. The DNA duplexes were incubated for 1 h at 37°C with 10 μM TFO or control oligonucleotide, as indicated, in a buffer consisting of 10 mM Tris-HCl (pH 7.4), 10% sucrose, and 5 mM MgCl2. After electrophoresis, the samples were transferred onto nylon membrane, and labeled DNA was detected using digoxigenin-specific chemiluminescent detection reagents.

In vitro binding of the TFO to the MCP-1 promoter

To assess the ability of the TFO to form triple helices with the target site in the MCP-1 promoter, gel mobility shift assays were performed. A synthetic 39-bp promoter fragment (Fig. 1) spanning the TFO target sequence and flanking regions was synthesized and end labeled with digoxigenin to allow detection. The promoter fragment was incubated with 10 μM TFO or control oligonucleotide (a scrambled oligonucleotide of the same base composition) in the presence of 5 mM MgCl2 and subjected to native polyacrylamide gel electrophoresis. Under the conditions used, triple helical complexes are stable during the course of the electrophoresis run (2 h) and migrate significantly slower than the corresponding duplex target DNA at physiological pH (19).

Results

Design of the TFO

A computer search was conducted to identify potential triple helix-forming target sites within the human MCP-1 gene using the sequences reported for the mRNA and the 5′ genome region in the GenBank database (26, 27). The sequences were scanned for motifs consisting of a continuous stretch of at least 15 purine bases in one strand, with no more than one mismatch (pyrimidine), and a minimum G content of 65%. One possible target site consisting of 19 bp was identified in the promoter region of MCP-1 at bp −66 to −48 (Fig. 1). The TFO target site in the MCP-1 promoter includes the binding site for the transcription factor SP-1 and partially overlaps a putative AP-1 binding site; both are sites that have been implicated in the regulation of MCP-1 gene expression. Since it has previously been shown that triple helix formation can interfere with transcription factor-DNA interactions, we anticipated that this might be a suitable target site for a TFO aimed at the inhibition of gene transcription. A triplex-forming oligonucleotide based on the antiparallel purine motif was designed according to the known rules, e.g., T opposite A:T pairs and G opposite G:C pairs (19). T was placed opposite the C-G inversion, because this base was previously shown to be tolerated opposite C:G pairs in triple helix motifs without adverse effect on the binding affinity of the TFO (28, 29). The purine motif was chosen, because purine oligonucleotides, unlike pyrimidine oligonucleotides, can bind to the target DNA at physiological pH (19).
Phosphorothioated oligonucleotides are much more stable to cellular nucleases than the phosphodiester oligonucleotides. In addition, they may have advantages for triple helix formation in cellular environments, because their interaction with DNA targets appears to be less sensitive to physiological concentrations of potassium ions (31). The phosphorothioated oligonucleotide also formed triple helices in a dose-dependent manner (Fig. 3C), with an EC$_{50}$ value of $1.5 \times 10^{-6}$ M (Fig. 3D).

**Inhibition of transcription factor binding by TFOs**

Because the TFO binding site in the MCP-1 promoter spans the entire binding site for the transcription factor SP-1, it was determined whether the binding of the TFO could interfere with transcription factor binding in vitro. The digoxigenin-labeled MCP-1 promoter duplex was incubated with the TFO and/or rSP-1, and the resulting DNA-DNA and DNA-protein complexes were analyzed by in vitro gel mobility shift assays (Fig. 4A). rSP-1 bound to the promoter duplex, and the interaction was inhibited in the presence of the TFO. The binding of cellular transcription factors was also analyzed. Nuclear extract proteins from unstimulated or TNF-$\alpha$-stimulated HEK 293 cells were incubated with the digoxigenin-labeled MCP-1 promoter duplex that had or had not been pretreated with the TFO. Under the assay conditions used, no DNA-protein complexes were observed when the DNA was incubated with nuclear extract proteins from unstimulated cells; however, two DNA-protein complexes were detected following incubation of the promoter DNA with TNF-$\alpha$-stimulated nuclear extract. In the presence of the TFO, the formation of both complexes was markedly reduced. We have tentatively identified SP-1 as one component of the DNA-protein complexes, because disruption of the SP-1 binding motif in the synthetic promoter duplex prevented the formation of the complexes; in contrast, mutation of the AP-1 binding site did not affect DNA-protein complex formation (data not shown).

**Inhibition of the expression of endogenous MCP-1 in cultured cells**

Our next aim was to test whether the TFO can inhibit expression of MCP-1 in cultured cells. Several human cell lines were initially screened by ELISA for cytokine-induced secretion of chemokines. We then selected human endothelial cells (ECs), because they express MCP-1 constitutively and are sensitive to the stimulation by TNF-$\alpha$.

**Inhibition of transcription factor binding by triple helix formation in vitro.** A, The digoxigenin-labeled MCP-1 promoter fragment described in Fig. 1 was incubated with 4 footprinting units, as defined by the supplier, of human rSP-1 in the presence or absence of 10 $\mu$M TFO. Incubation was for 1 h at 20°C in a buffer consisting of 10 mM Tris-HCl (pH 7.4), 10% sucrose, 20% glycerol, 1 mM DTT, 4 mM HEPES, and 5 mM MgCl$_2$. The samples were electrophoresed through 7% native polyacrylamide gels and transferred to nylon membrane, and labeled DNA was detected using digoxigenin-specific chemiluminescent detection reagents. A–C, One representative experiment of at least two for each oligonucleotide. D, Graphic representation of the titration curves, in which the data from all experiments were combined.
HEK 293 cells were found to produce low basal concentrations of MCP-1 and IL-8, and they could be induced by proinflammatory cytokines to secrete higher levels of these two chemokines (Fig. 5); IL-6 could not be detected in the supernatants of HEK cells (the detection limit was 25 pg/ml). The best stimulus for the induction of MCP-1 in HEK 293 cells was TNF-α, which increased the amount of secreted MCP-1 7-fold. HEK 293 cells can therefore serve as a model system to study MCP-1 gene expression, and this cell line was chosen for additional experiments.

HEK 293 cells were treated with various concentrations of phosphorothioated TFO for 48 h. To increase the resistance of the oligonucleotides to cellular nucleases and to reduce the dependence of the TFO-DNA interaction on K⁺ ions, the oligonucleotides with a phosphorothioate backbone were used. The oligonucleotides were added directly to the culture medium without the help of transfection reagents, because of earlier observations that the stress caused by mock transfections alone is sufficient to induce the expression of chemokines. Pretreatment of HEK 293 cells with the TFO for 48 h inhibited the secretion of MCP-1, but not IL-8, in unstimulated and TNF-α-stimulated cells, as determined by ELISA (Fig. 6). At 2 μM TFO, the MCP-1 secretion in unstimulated cells was reduced by 11%; in TNF-α-stimulated cells by 23%. At 5 μM, the effect was more pronounced; MCP-1 secretion from unstimulated cells was again lowered by 11% and secretion by TNF-α-stimulated cells was reduced by 45%. The secretion of IL-8 was unaffected at 2 or 5 μM TFO, indicating that the effects of the TFO were not caused by nonspecific inhibition of protein synthesis, but appear to be gene specific. The control oligonucleotide used had no effect on MCP-1 or IL-8 secretion at concentrations up to 5 μM. Higher concentrations of the TFO or control oligonucleotide resulted in decreased cell viability with concomitantly decreased secretion of both MCP-1 and IL-8 (data not shown). Treatment of the cells with 5 μM TFO for 24 h only did not result in significant reduction of MCP-1 secretion, indicating that oligonucleotide uptake and/or target binding in cells are slow processes.

To investigate whether the effects on MCP-1 secretion were caused by reduced gene transcription, MCP-1 expression was analyzed at the mRNA level. HEK 293 cells were treated with 5 μM TFO or control oligonucleotide for 48 h, then the cells were treated with TNF-α for 3 h and total RNA was isolated. The MCP-1 gene expression was analyzed by Northern analyses; a typical gel is shown in Fig. 7A. Densitometric analysis of three similar gels showed that the expression of MCP-1 mRNA in TNF-α-treated cells was reduced by ~40% by the TFO pretreatment compared with cells without oligonucleotide addition (Fig. 7B). Pretreatment with the control oligonucleotide resulted in a statistically nonsignificant 18% increase of MCP-1 mRNA. The effect of the TFO on the MCP-1 mRNA in unstimulated HEK 293 cells could not be determined, because in unstimulated cells the mRNA level was at or below the detection limit.

**Discussion**

The present investigation shows that a TFO is capable of in vitro binding to the SP-1 site of the human MCP-1 gene promoter. This interaction appears to be gene specific, because the oligonucleotide did not bind to the SP-1 region of the IL-6 gene. Binding of the oligonucleotide replaces the binding of nuclear proteins to the same site, including the transcription factor SP-1. It was further demonstrated that it is possible to use the oligonucleotide to partially inhibit the secretion of MCP-1 from HEK 293 cells. A reduction of MCP-1 mRNA was achieved by the TFO treatment, suggesting that the inhibitory effect on MCP-1 secretion was caused at the transcriptional level. The data have implications for the long-range goal to assess the feasibility of developing oligonucleotide-based anti-inflammatory agents that specifically reduce the expression of MCP-1 or other chemokines in vivo.

Previously, TFOs targeted to the IL-2R, TNF-α, GM-CSF, human mitochondrial aldehyde dehydrogenase, and several other genes have been described, with the observed EC₅₀ values for in vitro triplex formation ranging from 3 × 10⁻¹⁰ M to >10⁻⁵ M (20–22, 31). The EC₅₀ values for the TFOs described in this study fall within this range. The observation that the phosphorothioated oligonucleotide had an 8-fold higher EC₅₀ than the phosphodiester
The proteins in the nuclear extract that bind to the promoter fragment are not entirely clear. Based on preliminary results with promoter fragments containing mutations in the SP-1 or AP-1 binding sites, we have seen evidence for the participation of SP-1, but not AP-1, in the complexes; additional unidentified proteins may be present. This is consistent with previous reports that the AP-1 binding site is important in a broad range of cell types, whereas the AP-1 site in the promoter fragment regulates cytokine-induced MCP-1 expression in some but not all cell lines tested (23, 34).

The observation that the promoter-binding proteins were present in nuclear extracts only after TNF-α stimulation is not necessarily inconsistent with the involvement of SP-1. In the past, SP-1 has been thought of as a factor that is mainly associated with basal transcription, but there is increasing evidence now that SP-1 can be involved in the regulation of cytokine- and shear stress-induced transcription as well (35, 36). In any case, these in vitro findings support our hypothesis that triple helix formation at the MCP-1 promoter can serve as a mechanism for transcriptional repression by replacing or preventing the binding of necessary transcription factors.

In the tissue culture experiments, a partial inhibition of TNF-α-induced MCP-1 expression was seen; the observed inhibition levels are consistent with those reported by other laboratories for other TFO target genes in tissue culture. In one instance, up to 90% inhibition was achieved (31), but inhibition levels ranging from 14%–60% in cell culture have been more common. It is possible that to achieve quantitative inhibition of TNF-α-induced MCP-1 gene expression, other regulatory elements of the promoter, such as an NF-κB binding site at bp −2613/−2603, would have to be targeted as well; this cis element is essential for TNF-α- and IL-1β-induced enhancer activity in a number of cell types (23, 34).

It is also possible that the inhibition levels seen in cell culture were limited by the rate and extent of oligonucleotide uptake into the cells. Oligonucleotides have little ability to passively diffuse across cell membranes. They are taken up primarily through endocytosis, which is mediated in part by receptor-like oligonucleotide-binding proteins on the cell surface (37). After internalization, the oligonucleotide has to escape the vesicles and enter the cytoplasm intact. From the cytoplasm they reach the nucleus most efficiently by passive diffusion through the nuclear pore complex, an aqueous channel of ~9 nm in diameter that allows unhindered passage of molecules up to 40–60 kDa (38). Different cell types vary widely in their ability to endocytose oligonucleotides; it is also clear that depending on cell type there are more or less efficient efflux mechanisms leading to the elimination of oligonucleotides from the nucleus (20, 38). The relatively long incubation period of 48 h required for the TFO to be effective indicates that the accumulation of effective oligonucleotide concentrations in the nucleus may be a slow process.

It is noteworthy that the inhibition of MCP-1 secretion was significantly more pronounced in the TNF-α-stimulated cells than in the unstimulated cells (45% vs 11%, respectively, at 5 μM TFO). This latter observation might be related to differences in the accessibility of the target sequence under different stimulation conditions. For the TFO to bind to the target sequence, it must overcome some steric hindrance by the chromatin structure in the gene, which can undergo major changes upon up-regulation of transcription. The hypothesis that the TFO target region in the MCP-1 gene can change accessibility under cytokine stimulation is supported by a recent study that showed that IFN-γ-induced signaling resulted in changes in the genomic footprinting pattern of the MCP-1 promoter SP-1 binding site in astrocytoma cells (35).

To date, the successes achieved with TFOs in cell culture have not translated into TFO applications in vivo models. To achieve oligonucleotide of same sequence was unexpected. Hacia et al. (32) examined in detail the effects of changing the backbone from phosphodiester to phosphorothioate for two triplex target sites, and observed similar affinities for oligonucleotides in the antiparallel purine motif regardless of the backbone. The effects of the oligonucleotide backbone on the in vitro binding affinity may vary with sequence composition.

A comparison of the published data on different TFO targets shows that the EC50 values determined in binding assays are only of limited value for predicting the concentrations required to achieve effects in cell culture. Nevertheless, the in vitro binding assay can be a rapid and meaningful assay to compare the affinities of different probes for a given site and thus aid in the development of tighter binding analogues (25). For example, substituting some of the guanosine residues with 6-thioguanosine has been reported to increase the stability of the triplexes and to lower the sensitivity to K+ ions in some instances (33). Oligonucleotides with nonionic backbones are also predicted to have improved binding characteristics due to the elimination of electrostatic repulsion, and they have the added advantage of being resistant to cellular nucleases; these backbone modifications include methylphosphonates and phosphoramidates (19). Finally, the use of a nonnatural base analogue in the position binding to the C:G inversion could potentially improve the overall binding affinity of the TFO. These and other modifications of the TFO and observation of their effects on triplex formation represent an interesting area for future studies.

Occupancy of the SP-1 binding site by the TFO prevented the in vitro binding of SP-1 to the same site; thus, binding of either the TFO or of SP-1 to the promoter are mutually exclusive events. The binding experiments with the nuclear extract proteins from HEK 293 cells also showed that the binding of oligonucleotide or protein to the DNA target are competing reactions. The identities of

FIGURE 7. Inhibition of MCP-1 gene transcription by the TFO. HEK 293 cells were treated with 5 μM TFO or control oligonucleotide or left untreated, as indicated, for 48 h. Then the cells were left unstimulated or stimulated with TNF-α (500 U/ml) for 3 h. Total RNA was isolated from the cells, and 10 μg of RNA was subjected to formaldehyde-agarose gel electrophoresis, transferred to nylon membrane, and hybridized with digoxigenin-labeled RNA probes specific for MCP-1 or GAPDH. Detection of the labeled probes was performed with digoxigenin-specific chemiluminescence detection reagents. A. Shows one representative experiment of three. B, The TNF-α-stimulated samples were analyzed by densitometry. The relative OD of the MCP-1 bands were normalized using the GAPDH bands as control. The RNA level without oligonucleotide treatment was then defined as 100% and used as reference for the oligonucleotide-treated samples. The graph shows the mean values of all three experiments ± SEM.
this end, another area of intense research, besides the earlier mentioned efforts to develop base analogues with improved binding affinity, is the development of improved oligonucleotide delivery systems. Potentially interesting delivery systems in this regard could consist of the recently described penetrains, which are reported to function as trans-oligonucleotides for the intracellular delivery of DNA (39), or of polylysine-conjugated oligonucleotides in the presence of the capsid of a replication-deficient adenovirus (40). These systems can be readily studied in cell culture models such as the one we described, and insights gained by these studies might provide useful information for the development of novel in vivo delivery systems. This study, using the human MCP-1 gene as an example, supports the notion that, despite the current limitations of TFO technology such as limited number of genes with appropriate target sequences and variable accessibility of the target sequences in living cells, TFOs can be used in selected cases to specifically inhibit gene expression. It is the first report showing that the cytokine-induced synthesis of a chemokine can be influenced by TFO treatment in cell culture. Given the importance of MCP-1 in the progression of inflammatory diseases, such as glomerulonephritis or rheumatoid arthritis, it is imaginable that the ability to block the synthesis of this chemokine in vivo during an inflammatory reaction could have a beneficial effect on the course of these diseases. Thus, the further development of oligonucleotide-based reagents with improved binding affinities and/or delivery systems could be an alternative therapeutic strategy to MCP-1 receptor antagonists.

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