Oligodeoxynucleotides Enhance Lipopolysaccharide-Stimulated Synthesis of Tumor Necrosis Factor: Dependence on Phosphorothioate Modification and Reversal by Heparin

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ABSTRACT

Background: Specific inhibition of target proteins by antisense oligodeoxynucleotides is an extensively studied experimental approach. This technique is currently being tested in clinical trials applying phosphorothioate-modified oligonucleotides as therapeutic agents. These polyanionic molecules, however, may also exert non–antisense-mediated effects.

Materials and Methods: We examined the influence of oligonucleotides on lipopolysaccharide (LPS)–stimulated tumor necrosis factor α (TNFα) synthesis in freshly isolated human peripheral blood mononuclear cells. Oligonucleotides (18 mer) with different degrees of phosphorothioate modification were studied.

Results: The addition of phosphorothioate oligonucleotides (5 μM) caused amplification of TNF synthesis of up to 410% compared with the control with LPS alone. Without LPS stimulation, phosphorothioate oligonucleotides did not induce TNF production. We demonstrate that the enhancement of LPS-stimulated TNF production by phosphorothioate oligonucleotides does not rely on the intracellular presence of oligonucleotides and is not mediated by LPS contamination. Partially phosphorothioate-modified oligonucleotides and unmodified oligonucleotides did not increase TNF synthesis. High concentrations of the polyanion heparin reversed the oligonucleotide-induced enhancement of TNF synthesis.

Conclusions: The data suggest that amplification of TNF synthesis may be caused by binding of the polyanionic phosphorothioate oligonucleotide to cationic sites on the cell surface. Such binding sites have been proposed for polyanionic glycoaminoglycans of the extracellular matrix, which have also been described to augment LPS-stimulated TNF synthesis. The present results are relevant to all in vitro studies attempting to influence protein synthesis in monocytes by using phosphorothioate oligonucleotides. The significance of our findings for in vivo applications of phosphorothioates in situations where there is a stimulus for TNF synthesis, such as in sepsis, should be elucidated.

INTRODUCTION

Antisense oligonucleotides have been termed informational drugs because of their unique potential to translate the molecular understanding of disease into new therapeutic agents. The principle of antisense technique is the sequence specific hybridization of complementary single-stranded oligonucleotides to target RNA thereby inhibiting translation of the target protein (for review see Refs. 1–5). The efficacy of antisense oligonucleotides against neoplasia (6–9), viral infections (10), and post-traumatic neointimal hyperplasia (11) has been established in preclinical models. A total of seven oligonucleotides have entered clinical trials aiming at treatment of acute and chronic myelogenous leukemia (12–14), human immunodeficiency virus (HIV) infection (15), cytomegalovirus retinitis in acquired immunodeficiency syndrome (AIDS) patients (clinical trial phase III [16,17]), and genital

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warts (18). No severe side effects have occurred in human studies to date, including those with systemic administration (12,15).

Despite numerous reports documenting the efficacy of antisense oligonucleotides in vitro and in vivo in inhibiting gene expression, complementary RNA binding is not their sole mechanism of action (2,19,20). Different mechanisms are responsible for non–antisense-mediated effects. They can be divided into sequence-specific (so-called aptamer effect) and non–sequence-specific binding of oligonucleotides to proteins. The latter is primarily based on a charge interaction. In addition, hybridization to unintended RNA targets occur. These mechanisms explain several examples of nonantisense effects: (i) stimulation of B lymphocytes by oligonucleotides containing the CpG dinucleotide (21); (ii) anti-adhesive effects (19,22); (iii) sequence-specific, but not antisense-mediated, inhibition of cell proliferation (22,23); and (iv) inhibition of viral infection at the level of adsorption, penetration, or uncoating (4).

Non–antisense-mediated activities may contribute to side effects of oligonucleotides in clinical studies. Nevertheless, these effects could also be beneficial under some circumstances. In fact, oligonucleotides reduce melanoma growth in a SCID-hu mouse model by their interaction with basic fibroblast growth factor (bFGF) (20). In the present study, we demonstrate a new non–antisense-mediated effect of phosphorothioate oligonucleotides, the enhancement of lipopolysaccharide (LPS)–stimulated tumor necrosis factor (TNF) synthesis.

MATERIALS AND METHODS

Cell Culture

Human peripheral blood mononuclear cells (PBMC) were isolated from blood of healthy fasting volunteers by Ficoll-Hypaque gradient centrifugation (24) as described previously in detail (25). As a modification of the protocol, centrifugation was performed in tubes containing a horizontal porous filter disc over the Ficoll layer (Leucosep tubes; Greiner, Frickenhausen, Germany), in order to facilitate layering of blood. Cells were suspended in RPMI 1640 culture medium (Biochrom, Berlin, Germany) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES (all from Sigma, Munich, Germany), 1% human albumin (Curasan, Kleinostheim, Germany), and 1% heat-inactivated human serum. All compounds were purchased endotoxin-tested. Cells, 100 μl, were seeded at a final concentration of 2.5×10^6/ml (37°C, 5% CO_2 and fully humidified air) in flat bottom 96-well microtiter plates (200 μl/well). Oligonucleotides and/or heparin (Liquemin N, 10 000 IE/ml heparin-sodium, preservant-free, from porcine mucosa, Hoffmann-La Roche AG, Grenzach-Whylen) were added in 100 μl of supplemented medium for final concentrations as indicated. TNF synthesis was stimulated by LPS (*Escherichia coli* 055:B5; Sigma) diluted to a final concentration of 10 ng/ml. After 20 hr, addition of trypan blue to selected wells showed 95–97% dye exclusion, indicating preserved cell viability. Incubation was stopped by freezing the samples at −70°C. Cells were further disrupted by completing three freeze-thaw cycles. TNF concentrations were determined in combined cell lysate and supernatant by specific radioimmunoassay. Results are reported as means of experimental duplicates, unless otherwise indicated.

Oligonucleotides

Oligonucleotides were synthesized by Eurogentec (Seraing, Belgium). Lyophilisized oligonucleotides were diluted in sterile endotoxin-free water and stored at −20°C. The sequences used in this study are shown in Table 1. Oligonucleotides were chosen to exhibit minimal self-complementarity (computer program: OLIGO version 4; National Biosciences Inc., Plymouth, MN). All sequences were compared for possible homology to any known nucleotide sequence by a computer search in the GENBANK DNA database (EMBO, Heidelberg, Germany) using the computer program GCG (Version 7; Genetics Computer Group, Madison, WI). Oligodeoxynucleotide (ODN) 7 (arbitrary numbering) is an 18-mer oligonucleotide complementary to nucleotides 796–813 of TNF mRNA spanning the initiation region (see Refs. 26 and 27 for numbering). ODN 7 contains no CpG-dinucleotide and no more than two neighboring C- or G-nucleotides, motifs which have been shown to confer a high rate of nonspecific oligonucleotide effects. In addition, we used the sequence termed ODN 19, which contains 15 mismatches in comparison to ODN 7 (but identical qantitative nucleotide composition). Melting temperatures based on nearest neighbor thermodynamic parameters (28) are similar. Sequences are shown

TABLE 1. Characteristics of the oligonucleotides used.

Name	Sequence	Number of Mismatches	Number of Phosphorothioate Linkages	Melting Temperature
ODN 7cm	5′-**CAT GCT TTC AGT GCT CAT**-3′	0	17	56°C
ODN 7pm	5′-**CAT** GCT TTC AGT GCT **CAT**-3′	0	4	56°C
ODN 7um	5′-CAT GCT TTC AGT GCT CAT-3′	0	0	56°C
ODN 19cm	5′-***TAC TGC AGG ATT CTC TTC***-3′	15	17	51°C

Nucleotides on either side of a phosphorothioate linkage are indicated in bold letters. Mismatched nucleotides are in italics. The melting temperatures are estimated by the nearest neighbor method. The G/C content for all four oligonucleotides is 56%. A, adenosine; G, guanosine; C, cytosine; T, thymidine.

in Table 1. ODN 7 was used completely phosphorothioate-modified (ODN 7cm), partially phosphorothioate-modified (ODN 7pm) and unmodified (ODN 7um). ODN 19 was used completely modified (ODN 19cm). In phosphorothioate oligonucleotides one oxygen atom of the phosphate group is substituted by a sulfur atom.

Radioimmunoassay for TNF

TNF was determined by specific radioimmunoassay as previously described (29). In order to rationalize sample processing, a 96-microtube plate system with single polypropylene tubes (Sarstedt, Nümbrecht, Germany) was used. The sample (50 μl) was added to 50 μl of diluted polyclonal anti-TNF rabbit antiserum and 50 μl 1% rabbit IgG and was incubated overnight. Bolton Hunter-labeled ^{125}I-TNF (50 μl) (NEN/DuPont, Munich, Germany) was added on the second day. After another overnight incubation, 250 μl of second antibody (sheep anti-rabbit IgG) in 6% polyethylene glycol was added. TNF concentrations were calculated from a standard curve of human recombinant TNF (supplied by the National Institute for Biological Standards and Control, Potters Bar, United Kingdom), ranging from 0.02 to 10 ng/ml. The presence of oligonucleotide or heparin did not influence the measurement of TNF.

Endotoxin Assay

To exclude contaminations with endotoxin, all oligonucleotides were tested in the limulus amoebocyte lysate assay (Chromogenix, Charlston, SC, U.S.A.) and were found endotoxin negative (endotoxin content less than 6.0 pg/ml).

Statistical Analysis

Results are given as means ± SEM. The paired two-tailed Student's t test was performed for comparisons of means of TNF values. Differences were considered statistically significant for $p < 0.050$. Statistical analyses were performed by using Stat-View 512 software (Abacus Concepts, Calabasas, CA, U.S.A.).

RESULTS

Concentration-Dependent Enhancement of LPS-Stimulated TNF Synthesis by Phosphorothioate Oligonucleotides

Human mononuclear cells stimulated with 10 ng/ml of LPS for 20 hr synthesized 6.5 ng/ml TNF. Addition of increasing concentrations of phosphorothioate oligonucleotides led to enhanced TNF synthesis. Maximal TNF synthesis (up to 410% of control) was found for 2.5 and 5 μM ODN 7 (Fig. 1). Higher concentrations showed reduced efficacy in augmenting LPS-stimulated TNF synthesis. As low as 0.078 μM was effective. Subsequent experiments were performed using the concentration of 5 μM oligonucleotide.

Enhancement of TNF Synthesis Is Dependent on the Degree of Phosphorothioate Modification

The phosphorothioate modification alters the molecular structure of oligonucleotides. Despite its advantages over unmodified oligonucleotides with respect to stability, this modification in particular has been described to cause nonantisense effects. We studied the influence of the degree of

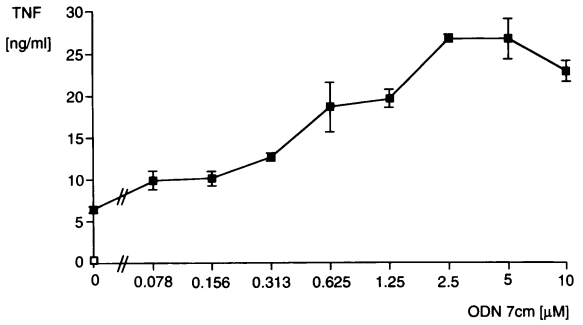

FIG. 1. Enhancement of LPS-stimulated TNF synthesis by phosphorothioate oligonucleotides

Human mononuclear cells (2.5 Mio/ml) were stimulated with 10 ng/ml LPS in the presence of the completely phosphorothioate-modified oligonucleotide ODN 7cm (0.078–10 μM). After 20 hr of incubation, TNF was measured using a specific RIA. Increasing concentrations of the oligonucleotide enhance TNF synthesis up to 410% of the control (▨). Without LPS stimulation (□), TNF synthesis was below the detection limit of the RIA. Data are shown as means of duplicates of a representative experiment.

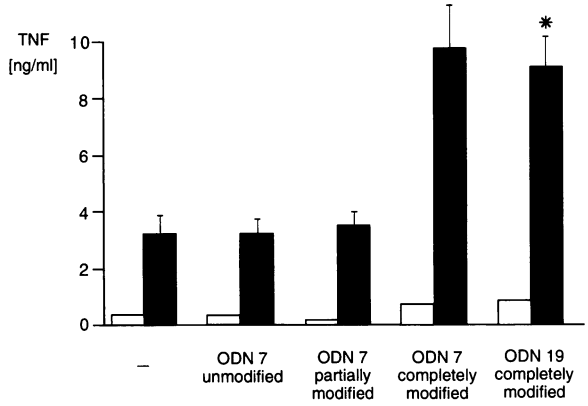

FIG. 2. Enhancement of TNF synthesis is dependent on the degree of phosphorothioate linkages and independent of the sequence

Human mononuclear cells (2.5 Mio/ml) were stimulated with 10 ng/ml LPS for 20 hr in the presence of the oligonucleotides indicated (all at 5 μM). No difference of TNF synthesis was found between the control with LPS alone (■) and in the presence of the partially modified oligonucleotide ODN 7pm (two phosphorothioate nucleotides at the 5′ and the 3′ end) and the unmodified oligonucleotide ODN 7um. LPS-stimulated TNF synthesis was enhanced only for the completely modified oligonucleotides ODN 7cm and ODN 19cm. Without LPS stimulation (□) only small induction occured in the presence of the completely modified oligonucleotides ODN 7cm and ODN 19cm, whereas TNF synthesis was at the detection limit in the presence of the partially and unmodified ODN 7. Results are given as means of three independent experiments. $*p < 0.05$; error bars = SEM.

phosphorothioate modification on enhancement of LPS-stimulated TNF synthesis.

The oligonucleotide ODN 7 was compared in three different forms: completely phosphorothioate-modified (ODN 7cm), partially modified (ODN 7pm) and unmodified (ODN 7um), each with identical nucleotide sequence. Neither the oligonucleotide with two phosphorothioate nucleotides at the 5′ and the 3′ end nor the unmodified oligonucleotide led to an increase in TNF synthesis in comparison to the control with LPS (Fig. 2). However, at the same concentration the completely modified oligonucleotide (5 μM) showed a reproducible enhancement of TNF synthesis to a mean of 290%. Without LPS stimulation, little TNF was induced by completely modified oligonucleotides (mean 0.78 ng/ml TNF), and still less by partially modified and unmodified oligonucleotides (at the detection limit of the RIA; i.e., 0.20 ng/ml TNF).

Enhancement of TNF Synthesis Is Not Dependent on the Sequence of the Oligonucleotide

Both sequence-dependent and sequence-independent nonantisense effects have been described for phosphorothioate oligonucleotides. We examined the role of the nucleotide sequence regarding the capacity of oligonucleotides in augmenting LPS-stimulated TNF synthesis.

The sequence complementary to the start site of TNF-mRNA (ODN 7) was compared with a mismatched control (ODN 19, same nucleotide content as ODN 7). In the presence of ODN 7cm, LPS-stimulated TNF synthesis was enhanced from 3.3 ng/ml (LPS stimulation alone) to 9.8 ng/ml. Despite the TNF sequence specifity of ODN 7, no significant difference in TNF amplification was found between ODN 7cm (mean 300%) and ODN 19cm (mean 280%; Fig. 2, right bar). No sequence-specific suppression of TNF synthesis could be achieved by applying five other TNF-specific sequences in this experimental system (data not shown).

Time Dependence of Enhanced TNF Synthesis by Phosphorothioate Oligonucleotides

Since partially and unmodified oligonucleotides show no difference in TNF production from the

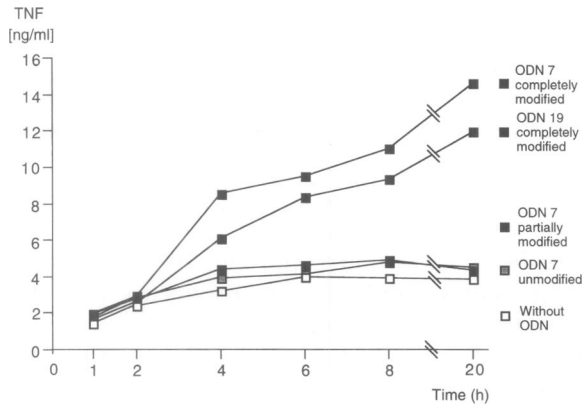

FIG. 3. Enhancement of TNF synthesis by phosphorothioate oligonucleotides is time dependent

Human mononuclear cells (2.5 Mio/ml) were stimulated with 10 ng/ml LPS in the presence of oligonucleotides added (5 μM). TNF synthesis was measured at different time points. In the presence of completely phosphorothioate-modified oligonucleotides (ODN 7cm and ODN 19cm) TNF synthesis was enhanced at all time points 4 hr or more after LPS stimulation. No plateau of TNF production was reached up to 20 hr. In contrast, the partially modified oligonucleotide ODN 7pm and the unmodified oligonucleotide ODN 7um did not enhance TNF synthesis compared with stimulation with LPS alone.

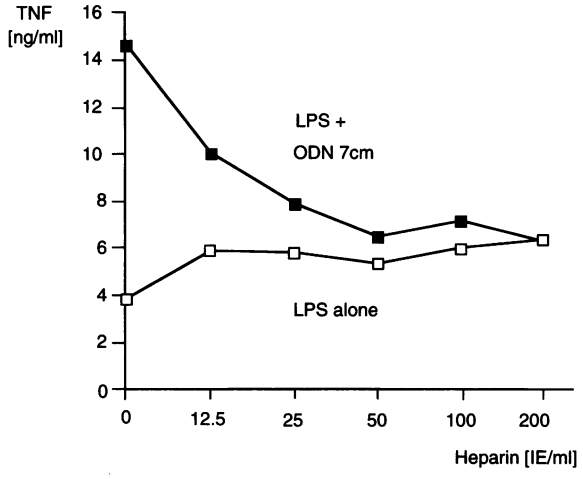

FIG. 4. Heparin reverses enhancement of TNF synthesis

Human mononuclear cells (2.5 Mio/ml) were stimulated with 10 ng/ml LPS for 20 hr. The influence of increasing concentrations of heparin on the enhancement of TNF production by phosphorothioate oligonucleotides (5 μM ODN 7cm) was tested. At a concentration above 25 IE/ml heparin reverses enhanced TNF synthesis to the level of the control with LPS alone. Reversal by heparin suggests displacement of oligonucleotides from the binding sites that mediate enhanced TNF synthesis. Influence of heparin on TNF measurement by the RIA was excluded.

control with LPS alone at 20 hr we investigated whether there was any difference at earlier time points. TNF synthesis was examined in the presence of oligonucleotides at consecutive time points (1, 2, 4, 6, 8, and 20 hr after LPS stimulation). In the presence of completely phosphorothioate-modified oligonucleotides (ODN 7cm and ODN 19cm), TNF synthesis was enhanced at all time points 4 hr or longer after LPS stimulation (Fig. 3). A plateau in TNF production was not reached for up to 20 hr. In contrast, the partially modified oligonucleotide ODN 7pm and the unmodified oligonucleotide ODN 7um did not enhance TNF synthesis compared with stimulation with LPS alone at all time points tested.

High Concentrations of Heparin Reverse Enhancement of TNF Synthesis by Phosphorothioate Oligonucleotides

A possible mechanism for the phosphorothioate oligonucleotide-mediated enhancement of TNF synthesis may be through their property of polyanionic macromolecules. We therefore investigated the influence of other polyanions on phosphrothioate oligonucleotide-enhanced TNF synthesis. Heparin is a ubiquitous, naturally occuring polyanionic polysaccharide, which is clinically used for its antithrombotic effect.

Mononuclear cells were stimulated in the presence of a completely modified oligonucleotide (ODN 7cm, 5 μM) and increasing concentrations of heparin (Fig. 4). The addition of heparin (12.5–200 IE/ml) without oligonucleotides led to a small increase of TNF production which was not concentration dependent. The TNF synthesis enhanced by phoshorothioate oligonucleotides was markedly reduced, from 14.7 ng/m to 10.1 ng/ml TNF, in the presence of 12.5 IE/ml heparin. Higher concentrations of heparin (50 IE/ml and more) completely reversed the enhancement of TNF synthesis by phosphorothioate oligonucleotides to the level achieved by LPS alone.

DISCUSSION

Antisense oligonucleotides were initially thought to have the unique potential of sequence-specific inhibition of single target proteins. Today, the

concept of absolute specifity has been replaced by the acceptance of nonantisense effects in addition to the antisense mechanism. Non–antisense-mediated immunological effects include the induction of interferon-γ and of IgG and IgM synthesis by oligonucleotides. These effects are dependent on defined motifs within the sequence (21). Numerous sequence-independent actions of oligonucleotides have also been reported in the literature (30–32). It has been proposed that nonspecific effects frequently predominate when phosphorothioate oligonucleotides are employed at concentrations higher than 5 μM (19).

In the present study, we describe the influence of phosphorothioate oligonucleotides on LPS-induced TNF synthesis in human mononuclear cells. We found an augmentation of TNF synthesis of up to 410% in the presence of 5 μM phosphorothioate oligonucleotide. Enhancement of LPS-stimulated TNF synthesis was concentration dependent, with a maximum between 2.5 and 5 μM. It was detectable for lower concentrations (down to 0.078 μM) than those proposed to be effective in the plasma of patients (12). In contrast to completely phosphorothioate-modified oligonucleotides, oligonucleotides with only two modified nucleotide bonds at each end or unmodified oligonucleotides showed no enhancement of TNF synthesis. The effect was independent of the sequence of the oligonucleotide.

The augmentation of TNF synthesis is not likely to be mediated by a direct action at the LPS receptor. We excluded relevant endotoxin contamination of the oligonucleotide preparations by using the limulus amoebocyte lysate assay. Furthermore, reversal of enhanced TNF synthesis by heparin argues against an effect of LPS.

The mechanism of the observed effect remains unknown. It likely relates to differences in the structures of modified versus unmodified oligonucleotides. In phosphorothioate oligonucleotides, one oxygen atom of the phosphate group is substituted by a sulfur atom. Phosphorothioate oligonucleotides represent polyanions with structural similarities to the naturally occuring polyanionic glycosaminoglycans of the extracellular matrix, such as heparan sulfate and heparin (Fig. 5). There are other examples of polyanionic macromolecules that enhance TNF synthesis in monocytic cells. Heparan sulfate increases LPS-stimulated TNF release from interferon-γ–treated THP-1 cells, a human monocytic cell line, by up to 500%. Chondroitin sulfate, keratan sulfate, and dermatan sulfate enhance LPS-stimulated TNF synthesis in THP-1 cells up to 200% (33). Chang et al. argued that glycosaminoglycans assist in the priming step of THP-1 cells, which is associated with enhanced TNF synthesis and secretion.

Hershkoviz et al. described other immobilized components of the extracellular matrix which induce TNF secretion in human mononuclear cells (34). The authors found the interaction between two glycoproteins of the extracellular matrix, laminin and fibronectin, and β1-integrins of the cell surface to be responsible for TNF production without requiring an additional specific stimulus. It has also been demonstrated that collagen stimulates the production and secretion of interleukin 1 in human mononuclear cells (35). Together, these results argue that the components of the extracellular matrix play an important role in potentiating cytokine synthesis by monocytes.

With regards to the binding of phosphorothioates to the surface of monocytes, it is of note that circulating human monocytes bind heparin in a rapid, saturable, and reversible manner (36,37). The affinity of heparin for the cell surface is independent of that for antithrombin III. In addition to sharing molecular features with heparin, phosphorothioate oligonucleotides have been described to bind directly to heparin-binding proteins, such as basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), FGF-4, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) (38). Oligonucleotides and sulfated glycosaminoglycans share binding sites on several other proteins. These include CD4, HIV-1 reverse transcriptase, the HIV-1 envelope glycoprotein and the protein kinase C β1-subunit (PKCβ1) (19). Since similar binding properties for heparin and oligonucleotides have been proposed, oligonucleotides may indeed bind to the surface of monocytes at the same sites as heparin.

Recently, another nonantisense mechanism mediated by the extracellular action of phosphorothioate oligonucleotides has been described (22). The non–sequence-specific inhibition of adhesion of glioblastoma cells could clearly be separated from the sequence-specific antisense effect. An excess of fibronectin, a glycoprotein of the extracellular matrix, restored the capability of the cells to adhere to the bottom of the culture plate. It was proposed that phosphorothioate oligonucleotides compete with molecules of the extracellular matrix such as fibronectin for binding sites at the cell surface. Both in that study and in the present experiments, the effect was abolished

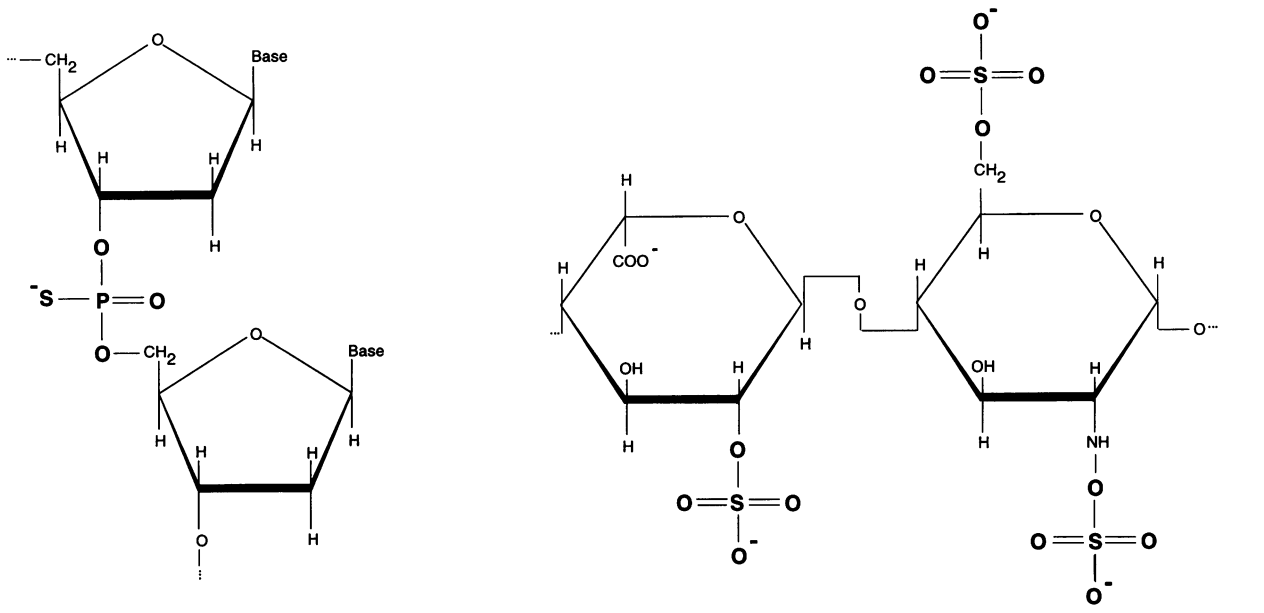

FIG. 5. Structural similarities between the polyanions phosphorothioate-deoxyribonucleic acid and heparin

In phosphorothioate-modified oligodeoxynucleic acid, one free oxygen atom of the phosphate is replaced by a sulfur atom, providing increased stability against nucleases. Base, the linkage to one of the bases adenosine, guanosine, cytosine, and thymidin. For heparin one of several possible sulfated disaccharides of the polysaccharide chain is shown. Both heparin and phosphorothioate-deoxyribonucleic acid represent polyanions. Groups of the molecules that are negatively charged under cell culture conditions are indicated with bold letters.

using oligonucleotides with a low degree of phosphorothioate modification or unmodified oligonucleotides. This suggests that the binding sites of phosphorothioate oligonucleotides that mediate the inhibition of adhesion and the increase in TNF synthesis are identical.

The priming monocytes by adherence results in cell activation (39). While adherence is sufficient to induce high steady-state levels of TNF mRNA in monocytes (40), actual translation and secretion of TNF requires the exposure to a second signal (39). As summarized above, glycosaminoglycans of the extracellular matrix are unable to induce TNF production by themselves, but promote TNF production when induced by a specific stimulus such as LPS. A biological role for this phenomenon may be that TNF synthesis is mainly restricted and strengthened in the extracellular compartment at the site of local inflammation. The underlying mechanism is likely to involve receptors that recognize components of the extracellular matrix. In the experiments described here, an excess of heparin reversed the enhancement of TNF synthesis by phosphorothioate oligonucleotides and restored levels similar to those achieved by LPS alone. Since phosphorothioate oligonucleotides and heparin are both polyanions and share molecular features, we propose that heparin displaces the oligonucleotides that mediate enhanced TNF synthesis from their binding sites on the cellular surface. Interestingly, heparin has been demonstrated to reduce the inflammatory response in vivo (41). One may speculate that the observed TNF-enhancing effect of phosphorothioate oligonucleotides, sharing molecular features with glycosaminoglycans of the extracellular matrix, represents a molecular mimicry of extravasal location.

Our findings are relevant to all in vitro studies attempting to influence protein synthesis in monocytes by phosphorothioate oligonucleotides. While we have examined LPS-induced

TNF synthesis, the enhancing effect may also occur using other stimuli such as phagocytized bacteria, and other monocyte-released proteins such as interleukin 1, interleukin 6, or tissue factor. If the enhancing effect is not accounted for, it may mask the intended inhibition of synthesis of a target protein. There are several experimental approaches to circumvent or minimize this non–antisense-mediated effect. First, as we have shown here, the effect can be minimized by reducing of the number of phosphorothioate-modified bonds within the oligonucleotide. This, in turn, has to be balanced with reduced stability of the oligonucleotide. Another approach, which we have shown in other studies (Hartmann et al., "Specific suppression of tumor necrosis factor α synthesis by antisense oligodeoxynucleotides," manuscript submitted), consists of removing oligonucleotide from the extracellular compartment through washing steps before stimulation with LPS. Finally, liposomal encapsulation of oligonucleotides facilitates penetration into the cells and may avoid nonantisense action of oligonucleotides in the extracellular compartment.

Whether the present findings are relevant to in vivo situations is not known. As phosphorothioate oligonucleotides alone did not induce TNF synthesis, one would not expect such an induction after systemic administration under ordinary conditions. In the case of exogenous or endogenous endotoxinemia (e.g., gram-negative sepsis), however, the possibility of enhanced TNF synthesis in the presence of oligonucleotides would have to be considered. In this context, it is of note that in monkeys a bolus injection of a dose higher than that proposed to be effective in humans of phosphorothioate oligonucleotides caused a lethal decrease of blood pressure (42).

Finally, this study adds to the existing evidence that sulfated polyanions enhance LPS-induced activation of monocytes. By analogy to glycosaminoglycans (33), fibronectin (34), and collagen (35), we have identified phosphorothioate oligonucleotides as a new class of macromolecules that participate in the priming of monocytes.

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