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Review

Decoy oligodeoxyribonucleotides and peptide nucleic acids–DNA chimeras targeting nuclear factor kappa-B: Inhibition of IL-8 gene expression in cystic fibrosis cells infected with *Pseudomonas aeruginosa*

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ABSTRACT

Cystic fibrosis (CF) is characterized by a deep inflammatory process, with production and release of cytokines and chemokines, among which interleukin 8 (IL-8) represents one of the most important. Accordingly, there is a growing interest in developing therapies against IL-8, with the aim of reducing the excessive inflammatory response in the airways of CF patients. Since transcription factor NF-kappaB plays a critical role in IL-8 expression, the transcription factor decoy (TFD) strategy might be of interest. TFD is based on biomolecules mimicking the target sites of transcription factors (TFs) and able to interfere with TF activity when delivered to target cells. Here, we review the inhibitory effects of decoy oligodeoxyribonucleotides (ODNs) on expression of IL-8 gene and secretion of IL-8 by cystic fibrosis cells infected by *Pseudomonas aeruginosa*. In addition, the effects of decoy molecules based on peptide nucleic acids (PNAs) are discussed. In this respect PNA–DNA–PNA (PDP) chimeras are interesting: (a) unlike PNAs, they can be complexed with liposomes and microspheres; (b) unlike oligodeoxyribonucleotides (ODNs), they are resistant to DNAses, serum and cytoplasmic extracts; (c) unlike PNA/PNA and PNA/DNA hybrids, they are potent decoy molecules. Interestingly, PDP/PDP NF-kappaB decoy chimeras inhibit accumulation of pro-inflammatory mRNAs (including IL-8 mRNA) in *P. aeruginosa* infected IB3-1, cells reproducing the effects of decoy oligonucleotides. The effects of PDP/PDP chimeras, unlike ODN-based decoys, are observed even in absence of protection with lipofectamine. Since IL-8 is pivotal in pro-inflammatory processes affecting cystic fibrosis, inhibition of its functions might have a clinical relevance.

Contents

Abbreviations: PNA, peptide nucleic acids; PDP, PNA–DNA–PNA chimeras; NF-kappaB, nuclear factor kappa B; I-kappaB, inhibitor of NF-kappaB; EMSA, electrophoretic mobility shift assay; CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; PAO-1, *Pseudomonas aeruginosa*, strain O1; PCR, polymerase chain reaction; RT-PCR, reverse transcription PCR; TF, Transcription factor; TFD, Transcription factor decoy.

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1. Introduction

Cystic fibrosis (CF) is a common genetic disease caused by mutations of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which encodes for a chloride channel expressed in several epithelia [1,2]. Defective CFTR causes chronic pathology in lungs, pancreas, liver, reproductive system, being the airway tract disease the most relevant cause of morbidity and mortality in CF [1–5]. The most important clinical complication in the airway tract of patients affected by cystic fibrosis is inflammation. This starts in the early phases of the disease, even in the absence of infection, as observed in the sterile bronchoalveolar lavage fluid collected in young CF infants, and is further amplified by recurrent bacterial infections (e.g. by *Haemophilus influenzae* and *Staphylococcus aureus*) and is followed by chronic bacterial colonization with *Pseudomonas aeruginosa* (*P. aeruginosa*) growing in mucoid biofilms of alginate during the advanced phases of the disease.

The chronic inflammatory response, starting early and proceeding throughout the whole life of patients affected by CF, is unable to eradicate bacterial infection from the conductive airways. The presence of bacteria amplifies the release of neutrophilic chemokines, such as interleukin-8 (IL-8) and GRO-α, and pro-inflammatory cytokines, such as IL-6 and IL-1β, in the airway mucosa making the presence of a huge amount of neutrophils a hallmark in CF [1,2]. Some of the biochemical and cellular features driving the expression of different cytokines and chemokines are shown in Fig. 1A. Importantly, pro-inflammatory cytokines, neutrophilic chemokines and adhesion molecules involved in chemotaxis, such as IL-6, IL-1β, IL-8, GRO-α/γ and ICAM-1, induced by *P. aeruginosa* in bronchial epithelial cell *in vitro*, are those found in the bronchoalveolar fluid of CF patients [6–8].

In conclusion, there is good consensus on the fact that lung inflammation is excessive in CF lung, and regulation of this process decreasing the undesired effects with novel anti-inflammatory strategies is relevant for therapy of cystic fibrosis [8]. Therefore, molecular strategies able to inhibit the expression of CF-associated cytokines and chemokines are of great interest. In this respect several *in vitro* experimental systems are available [9–14] one of which, constituted by the cystic fibrosis IB3-1 cell line infected with *P. aeruginosa*, is extensively used by our research group to identify possible agents useful for therapy of inflammation associated with CF [12–14].

2. Induction of pro-inflammatory genes in IB3-1 cystic fibrosis cells infected with *P. aeruginosa*

In order to mimic *in vitro* the induction of cytokines and chemokines found to be highly released in CF, the effects of infection by *P. aeruginosa* strain PAO1 of the expression of pro-inflammatory genes were analyzed in several studies [9–12]. For instance, when IB3-1 cells are infected by *P. aeruginosa* for 4 h and the content of RNAs coding for several pro-inflammatory proteins is analyzed by RT-PCR, the results summarized in Fig. 1 (panel B) are obtained, indicating that IL-8 mRNA content increases several folds in respect to basal levels of uninfected cells, assumed to be 1 (Fig. 1B). In addition, Fig. 1 shows that GRO-γ, GRO-α, IL-6, IL-1β,

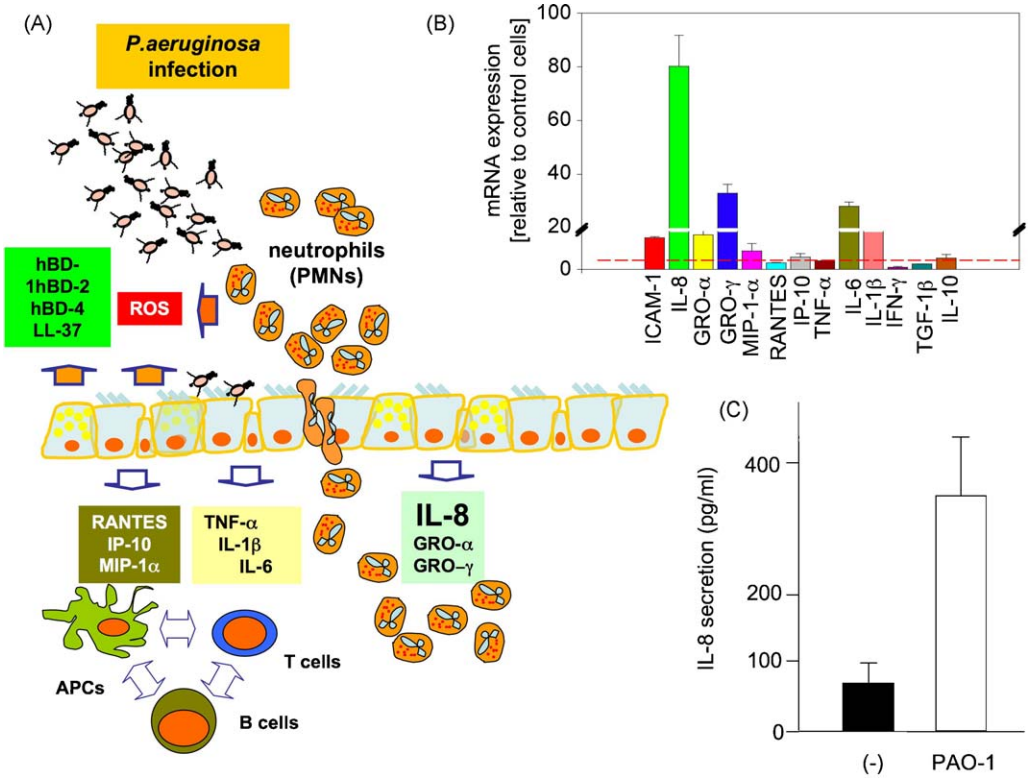

Fig. 1. (A) Early phases of cystic fibrosis lung infection and inflammation. The inflammatory hallmark of this and the following phases is an elevated secretion of the chemokine IL-8, which drives an abnormally elevated recruitment of polymorphonuclear neutrophils in the bronchial walls and lumina. Reduced mucociliary clearance in CF airways favors recurrent bacterial infections with motile bacteria, which further increase the release of chemokines and pro-inflammatory cytokines. In the advanced stages, a peculiarly and not fully explained selection of the Gram negative bacterium *P. aeruginosa* occurs. (B, C) Increased accumulation of mRNAs coding ICAM-1, IL-8, GRO-α, GRO-γ, MIP-1α, RANTES, IP-10, TNF-α, IL-6, IL-1β, IFN-γ, TGF-1β, IL-10 (B) and IL-8 secretion (C) in IB3-1 cystic fibrosis cells infected with *P. aeruginosa* strain PAO1. Total RNA from IB3-1 cells was reverse-transcribed to cDNA and the cDNA was then amplified by RT-qPCR. Primer sequences for detection of the indicated mRNAs have been reported in Bezzerri et al., 2008 [14]. Changes in mRNA expression level were calculated following normalization to the GAPDH calibrator gene. The ratios obtained following normalization are expressed as fold change over untreated samples (adapted from Bezzerri et al., 2008) [14]. In panel B, mRNA from control cells has been set as 1. For quantification of the release of IL-8, the Bio-plex technology was employed.

ICAM-1 mRNAs are also induced by PAO1, despite to an extent lower than IL-8 mRNA. On the contrary, no or very low increase of accumulation of IP-10, RANTES, MIP-1α, TNF-α, IFN-γ, TGF-β and IL-10 mRNA was observed under these experimental conditions [12,13]. Fig. 1C shows a representative experiment indicating a good relationship between the RT-PCR data (Fig. 1B) and the quantification of IL-8 secretion by PAO1 treated IB3-1 cells. Since the expression of several genes coding cytokines and chemokines involved in cystic fibrosis inflammation is under the control of several transcription factors [13–15], molecules targeting the biological functions of these regulatory proteins appears to be of great interest [13,14]. In this respect, the control of inflammation in cystic fibrosis is strictly related to NF-kappaB activation and targeting of NF-kappaB regulated genes [15–19].

3. The NF-kappaB superfamily of transcription factors

Several proteins belong to the NF-kappaB family, including RelA (also known as p65), RelB, cRel/Rel, p50 and p52, originating homo- and hetero-dimers, the most common of them being p50/p65 and p52/RelB. The NF-kappaB proteins are able to bind, together with co-activators, to specific DNA elements constituted by either a symmetric consensus (5′-GGG GAT TCC CCT-3′, such as that present on the Immunoglobulin IgK light chain promoter) or a non-symmetric sequence (5′-CGC TGG GGA CTT TCC ACG G-3′, such as that present within the long terminal repeat of the human immunodeficiency type 1 virus) [20–27]. The metabolic regulation of NF-kappaB biological functions involves several control levels, one of the most important being the interaction with inhibitory proteins belonging to the IkappaB (inhibitor of NF-kappaB) family [20,21]. Among these, IkappaBα plays a major role as recently reviewed by Ferreiro and Komives [20], generating a complex with the NF-kappaB homo- or hetero-dimers; this molecular interaction prevents NF-kappaB to translocate to the nucleus and exert its regulatory functions on transcription of target genes [20–22]. Activation of NF-kappaB is operated by the IkappaB kinase (IKK) complex, which is composed by the two catalytic subunits IKKα and IKKβ and a regulatory subunit, the NF-kappaB essential modulator (NEMO, also known as IKKγ) [28]. Upon different stimuli, IKK phosphorilates the N-terminal signal response domain of NF-kappaB-bound IkappaBα, causing subsequent polyubiquitinylation and proteasome-directed degradation, thus leading NF-kappaB to be translocated to the nucleus. At the nuclear level, NF-kappaB binds to the DNA target elements present in NF-kappaB regulated genes, as well as to co-activators of gene transcription [27].

4. NF-kappaB activity is inhibited by transcription factor decoy (TFD) molecules

The transcription factor decoy (TFD) approach [28–32] is based on the competition for trans-acting factors (TF) between endogenous cis-elements present within the regulatory regions of the target gene and exogenously added decoys molecules (for instance double-stranded DNA) mimicking the specific cis-elements [28–30]. This TFD strategy results in the attenuation of the authentic cis–trans interactions, leading to decreased binding levels of TF to endogenous cis-elements. The technique has been proven effective *in vitro* and *in vivo*, suggesting its use in therapy [31–41]. For instance, the TFD could be a very useful approach to alter the gene expression and develop anti-tumor agents [30]. In fact, it is well known that a variety of transcription factors are involved in neoplastic cell growth and tumor onset and development, such as Sp1, GATA-1, NF-Y, GATA-4 and GATA-6, NF-kappaB, CRE-binding proteins, Ets1, TTF-1, AP-1, AP-2, ERα. It should be underlined that the results of a decoy approach may be sharply different, depending on the biological role of the target TF. If the target TF is a direct or indirect activator of transcription, the TFD approach will lead to a very effective inhibition of transcription (Fig. 2A and B). On the contrary, if the TF target of the decoy approach is a

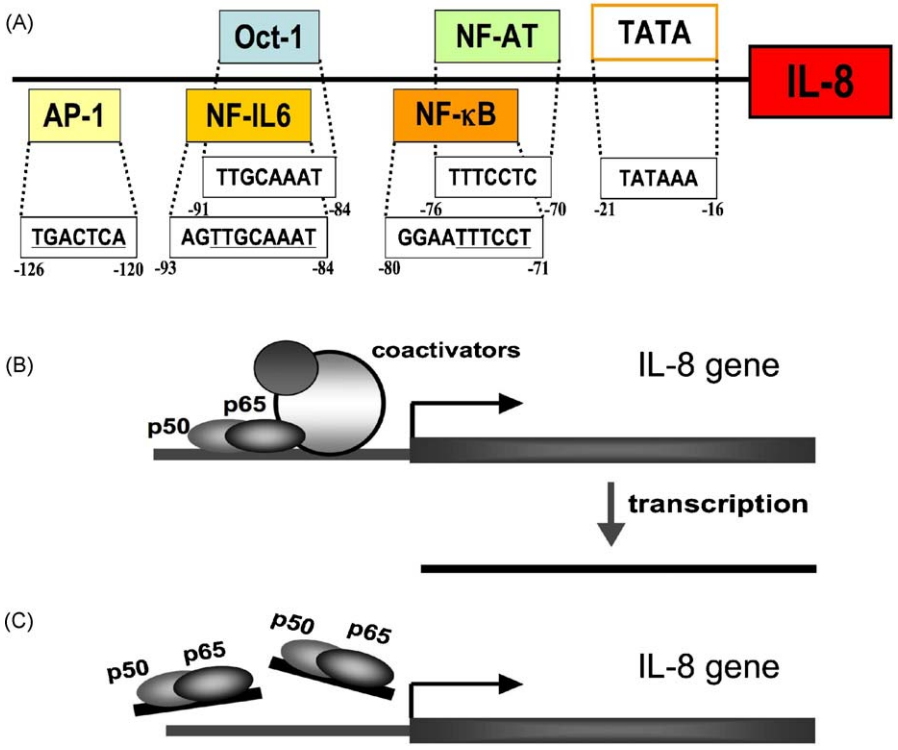

Fig. 2. (A) Binding sequences for transcription factors present in the promoter of the human IL-8 gene. (B, C) Expected effects of the TFD approach on the transcription of IL-8 gene. The transcription of IL-8 gene is dependent from NF-kappaB interactions (B); in the presence of decoy molecules, NF-kappaB is not able to efficiently interact with the IL-8 promoter and IL-8 gene transcription is inhibited (C).

transcription repressor, we expect a sharp induction of transcription in the decoy-treated cells.

A large number of reports support the concept that interference of NF-kappaB activity can be achieved using TFD molecules (43–49). This issue was reviewed by Morishita et al. [42], who compared the use of antisense, ribozymes, small interfering RNAs and transfection of cis-element double-stranded oligodeoxynucleotides with respect to inhibition of NF-kappaB activation. TFD molecules have been reported as a powerful tool in a new class of anti-gene strategies for molecular therapy. Isomura and Morita reviewed the application of NF-kappaB decoy ODNs to control inflammatory disorders and transplantation tolerance [42]. The conclusion of this and similar papers strongly support the concept that regulation of NF-kappaB signaling by NF-kappaB decoy ODNs might be an effective tool to control a variety of disorders caused by inflammatory immune responses [42–45]. Since NF-kappaB was clearly demonstrated to be a promising therapeutic target in inflammatory lung diseases [46,47], this issue was object of several investigations, in consideration of the fact that inflammatory lung disorders (such as asthma and chronic obstructive pulmonary disease) are responsible for significant morbidity and mortality worldwide. Edwards et al. [48] recently reviewed DNA oligonucleotides and DNA-peptide molecules acting as NF-kappaB decoy sequences as are very interesting molecules able to inhibit NF-kappaB functions involved in these two important inflammatory lung diseases.

In conclusion, a growing number of research papers and reviews support the concept that oligonucleotide decoys against NF-kappaB should be considered for altering the expression of NF-kappaB dependent genes, being therefore of interest for therapeutical approaches based on alteration of the NF-kappaB signaling.

5. Effects of decoy ODNs targeting NF-kappaB on gene expression of *P. aeruginosa* infected IB3-1 cells

In the experiments aimed to inhibit the activity of transcription factors, the CF bronchial IB3-1 cells have been transfected with TFD ODNs previously designed to mimic the DNA-binding site of NF-kappaB and tested in their capacity to interfere with the transcription of genes regulated by this TF. In order to test the ability of this decoy ODNs to compete for the binding of NF-kappaB to the sequences contained in the promoter of IL-8 gene (see Fig. 2C), Bezzerri et al. [14] incubated cold decoy ODNs with nuclear extracts from IB3-1 cells in the presence of a radiolabelled probe 100% homologous with the NF-kappaB binding sequences and performed electrophoretic mobility shift assays (EMSA). Complete inhibition of interaction of the ^{32}P-labelled probes with specific transcription factor proteins (NF-kappaB/DNA complexes) has been obtained, providing the proof of principle of the competition of this NF-kappaB decoy ODNs for the DNA consensus sequence contained in the promoter of the IL-8 gene [14]. Then, IB3-1 cells have been transfected with NF-kappaB ODNs complexed with cationic liposomes. The experimental strategy is shown in the upper part of Fig. 3 (panels A–C). Complexes of cationic liposomes with NF-kappaB or scrambled ODNs have been pre-incubated with IB3-1 cells 24 h before exposure to the PAO1 laboratory strain of *P. aeruginosa* (100 CFU/cell) for a further four hours time period. A scrambled ODN was used as negative control. After the treatment RNA was extracted and real-time quantitative RT-PCR performed. The results obtained demonstrated that the NF-kappaB decoy ODNs are efficiently internalized within the IB3-1 cells (Fig. 3D) and sharply inhibit accumulation of IL-8 mRNA (Fig. 3E).

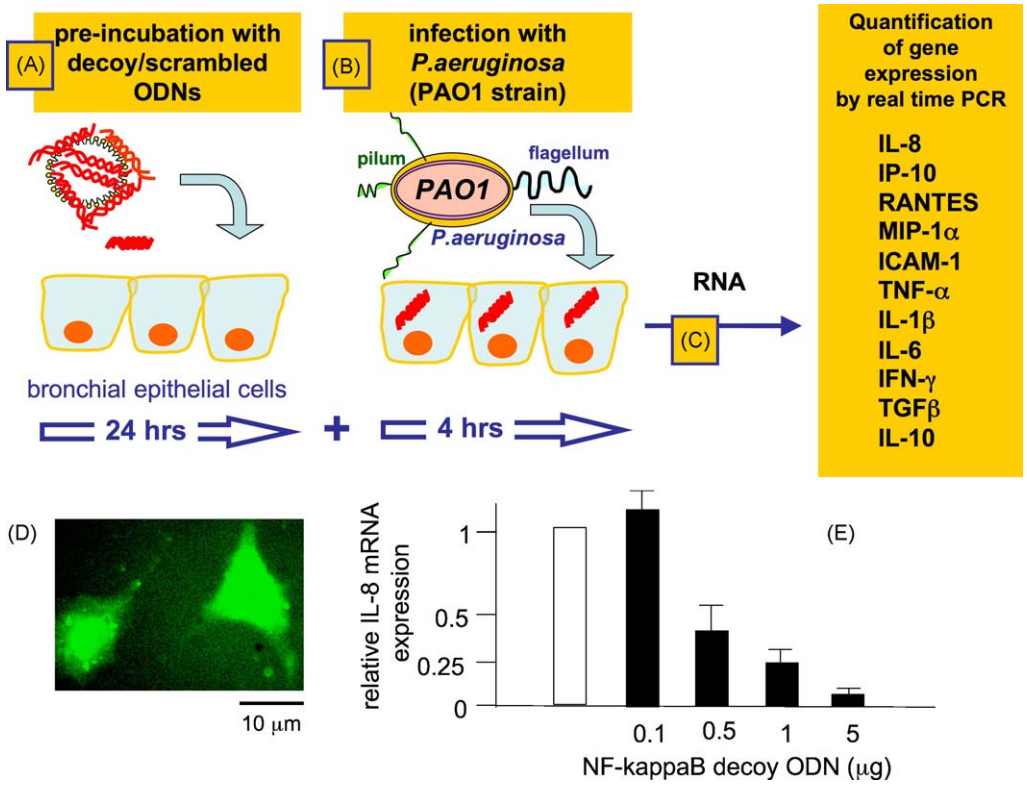

Fig. 3. (A–C) Experimental design followed for the analysis of the effects of the transcription factor decoy (TFD) strategy employing TF decoy ODNs against NF-kappaB and human bronchial epithelial cells (A–C). Human bronchial CF-derived respiratory epithelial IB3-1 cells were pre-incubated for 24 h with NF-kappaB decoys or scrambled ODNs (A) before infection with the laboratory strain of *P. aeruginosa* PAO1 (B). After 4 h post-infection, total RNA was extracted, reverse-transcribed to cDNA and analyzed by RT-qPCR (C). Primer sequences for detection of IL-8 mRNA were 5′-GAC CAC ACT GCG CCA ACA-3′ (IL-8 forward) and 5′-GCT CTC TTC CAT CAG AAA GTT ACA TAA TTT-3′ (IL-8 reverse). (D) Uptake of fluorescein-labelled TF decoy ODNs by IB3-1 cells. (E) Effects of NF-kappaB decoy ODNs on *P. aeruginosa*-dependent induction of IL-8, quantified as described in Fig. 1. Values are mean ± S.E.M. of four experiments.

6. Effects of NF-kappaB decoy ODNs: *in vivo* studies

Recent published reports demonstrate that NF-kappaB decoys are active *in vivo* [49–57]. For instance, NF-kappaB decoy oligonucleotides were demonstrated to ameliorate hepatic cold ischemia/reperfusion injury [49], reduce fatal liver failure after excessive hepatectomy [50], suppress nerve injury, mechanical allodynia, and thermal hyperalgesia in a rat lumbar disc herniation model [51], inhibit clinical symptoms related to periodontal diseases [52,53], is useful to prevent renal fibrosis in an animal model of nephropathy [54].

In respect to lung-associated diseases, decoy ODNs against NF-kappaB Kimura et al. demonstrated that these molecules, after nanoparticle-mediated delivery, are able to ameliorate monocrotaline-induced pulmonary arterial hypertension [55]. This and related studies demonstrated as very important point the delivery strategy of NF-kappaB decoys, as further demonstrated by two recent papers. In a first work, De Stefano et al. [56] investigated the potential of a delivery system able to improve cellular uptake and rapid *in vivo* enzymatic degradation of decoy ODN targeting NF-kappaB. They demonstrated that poly(D,L-lactide-*co*-glycolide) (PLGA) microspheres are able to efficiently increase the ODN stability in biological environment and release the encapsulated drug in long time frames. Interestingly, the decoy ODN against NF-kappaB was administered in naked form or was delivered by PLGA micropsheres in a rat model of chronic inflammation [56]. The subcutaneous implant of λ-carrageenin-soaked sponges caused leukocyte infiltration and formation of granulation tissue which were inhibited by co-injection of microspheres releasing decoy ODN. Naked decoy ODN showed the same effect, by only for few days [56]. Molecular analysis demonstrated an inhibition of NF-kappaB activation correlated to a decrease of tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS) expression. *In vivo* effects of decoy ODNs were also reported by Li et al. [57], who investigated whether NF-kappaB can be blocked by intratracheal administration of NF-kappaB ODNs, and whether decoy ODN mediated NF-kappaB inhibition can prevent smoke-induced lung inflammation, respiratory dysfunction, and improve pathological alteration in the small airways and lung parenchyma in the long-term smoke-induced mouse model system [57]. Analysis of respiratory function indicated that transfection of NF-kappaB decoy ODNs significantly impacted peak expiratory flow (PEF), and bronchoalveolar lavage cytology displayed evidence of decreased macrophage infiltration in airways compared to normal saline-treated or scramble NF-κB decoy ODNs smoke exposed mice. Li et al. [57] demonstrated that NF-kappaB decoy ODNs were able to significantly inhibit the level of macrophage inflammatory protein (MIP) 1α and monocyte chemoattractant protein 1(MCP-1) in lung homogenates.

The demonstration that NF-kappaB ODNs might exert anti-inflammatory responses *in vivo* is of great interest and further molecules should be considered, especially in consideration of the fact that that microspheres and/or liposomes are required to improve pharmacokinetics of decoy ODNs minimizing degradation [56]. In this respect, PNA-based molecules should be considered of interest in studies on artificial regulation of gene expression with the objective of inhibition of NF-kappaB activation in chronic inflammation.

7. Peptide nucleic acid (PNA)-based NF-kappaB transcription factor decoys

Peptide nucleic acids (PNAs) are DNA mimicking molecules in which the pseudopeptide backbone is composed of N-(2-aminoethyl)glycine units [58–64]. PNAs are resistant to both nucleases and proteases [64,65] and, more importantly, hybridize with high affinity to complementary sequences of single-stranded RNA and DNA, forming Watson–Crick double helices [58–61]. For these reasons, PNAs were found to be excellent candidates for antisense and antigéne therapies [65]. The possible use of peptide nucleic acids (PNAs) as alternative reagents in experiments aimed at the control of gene expression involving the decoy approach has been reported in several research papers and reviews, with the major limit of the low uptake by eukaryotic cells, a parameter very important for DNA-based treatment [66–71]. In a first study, Mischiati el al. [72] demonstrated that NF-kappaB p52 is able to bind to both NF-kappaB DNA/DNA and DNA/PNA hybrid mimicking the NF-kappaB target sites present in the HIV-1 LTR. Low binding of NF-kappaB p52 to PNA/PNA hybrids was on the contrary found [72,73]. Saviano et al. [73] used computational procedures to compare the behavior of PNA double-stranded molecules and PNA/DNA hybrids with the behavior of regular DNA duplexes in generating complexes with NF-kappaB, by performing molecular dynamics (MD) simulations *in vacuo*. The analysis performed clearly allowed them to predict that the lack of charged phosphate groups and the different shape of the helix play a critical role in the binding efficiency to NF-kappaB transcription factors [73]. In addition, the data obtained by Saviano et al. [73] confirmed that NF-kappaB binding proteins recognize both the NF-kappaB DNA/PNA and DNA/DNA hybrids, but the molecular complexes generated with NF-kappaB DNA/PNA hybrids are less stable than those generated with NF-kappaB target DNA/DNA molecules.

In more recent studies, PNA–DNA chimeras [74] have been described as reagents for the transcription factor decoy approach. PNA–DNA chimeras are PNA–DNA covalently bonded hybrids (see Fig. 4A) and were designed on one hand to improve the poor cellular uptake and solubility of PNAs, on the other hand to exhibit biological properties typical of DNA, such as the ability to stimulate RNaseH activity and to act as substrate for cellular enzymes (for instance DNA polymerases). The results published by Romanelli et al. [75], Borgatti et al. [76,77] and Moggio et al. [78] firmly demonstrate that decoy molecules based on PNA–DNA chimeras are powerful decoy molecules.

Romanelli and co-workers in 2001 employed the molecular modelling approaches to determine the three-dimensional structure of PNA–DNA–PNA (PDP) chimera, in order to predict their molecular interactions with NF-kappaB and explain the exciting finding that decoy molecules based on PNA–DNA chimeras are decoy molecules efficient as DNA–DNA decoys [75]. The structure of a double-stranded NF-kappaB PNA–DNA–PNA chimera was performed by energy minimization and molecular dynamics simulations. Fig. 4B shows the molecular model of the average structure of NF-kappaB PDP-PDP duplex as obtained from molecular dynamics (MD) simulation *in vacuo*. The data obtained indicate that the structure of the PNA–DNA–PNA molecule (Fig. 4B) is clearly very similar to that of the corresponding DNA–DNA double helix. In agreement, the data obtained by EMSA (the sequences of the employed decoy molecules are shown in Fig. 4, panels C and D; a representative EMSA analysis is shown in Fig. 5A and B) demonstrate that these PDP-based structures are very active decoy molecules.

By using RT-qPCR analysis we have demonstrated that (a) the effects of PDP/PDP NF-kappaB decoy chimeras on accumulation of pro-inflammatory mRNAs in *P. aeruginosa* infected IB3-1 cells reproduce that of decoy oligonucleotides; in particular (a) the NF-kappaB PDP/PDP chimera exhibits differential effects on expression PAO1 activated genes (Fig. 5B), such as ICAM-1, GRO-γ, IL-1β, IL-6, IL-8; (b) these PDP/PDP chimeras are strong inhibitor of IL-8 gene expression (Fig. 5C); (c) the effects of PDP/PDP chimeras, unlike those of ODN-based decoys, are observed even in the absence of protection with lipofectamine (Fig. 5C). While it is firmly established that the entry of oligonucleotides into

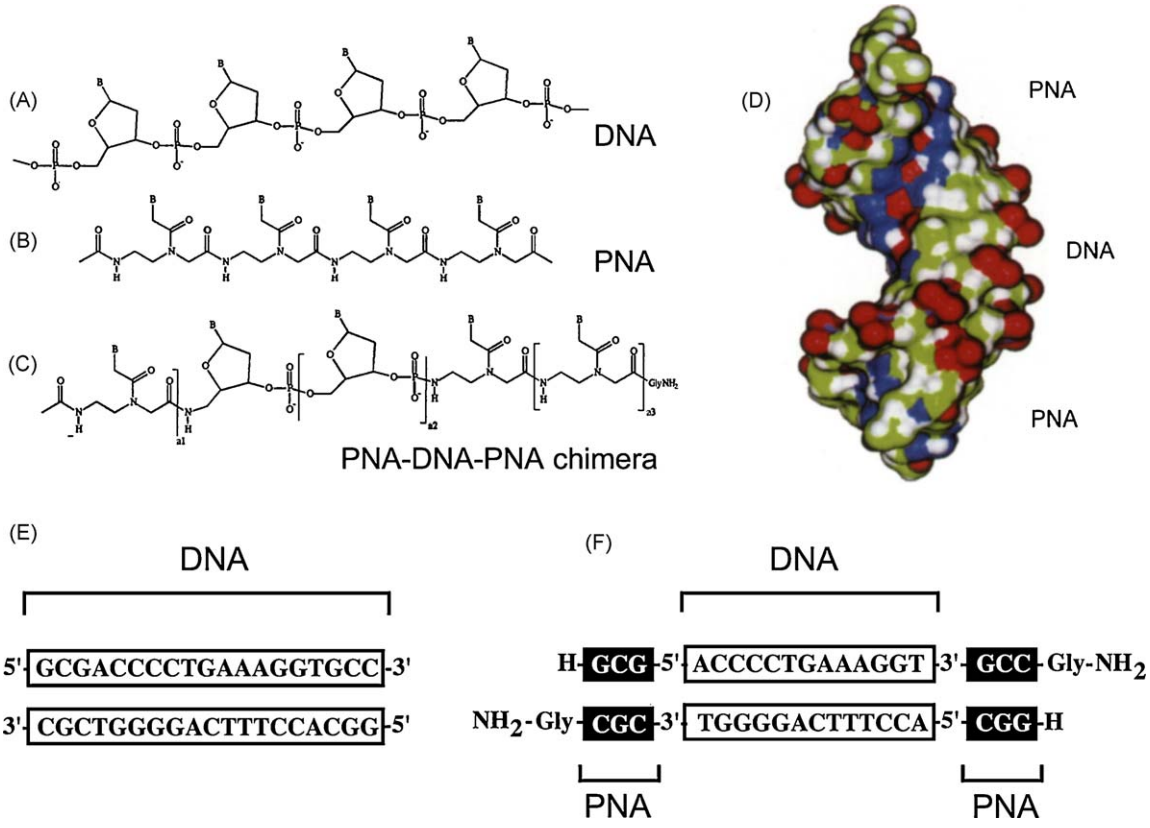

Fig. 4. Structures (A–D) and sequences (F) of the double-stranded PNA–DNA–PNA chimeras. The sequences of reference NF-kappaB ODN is shown in (E).

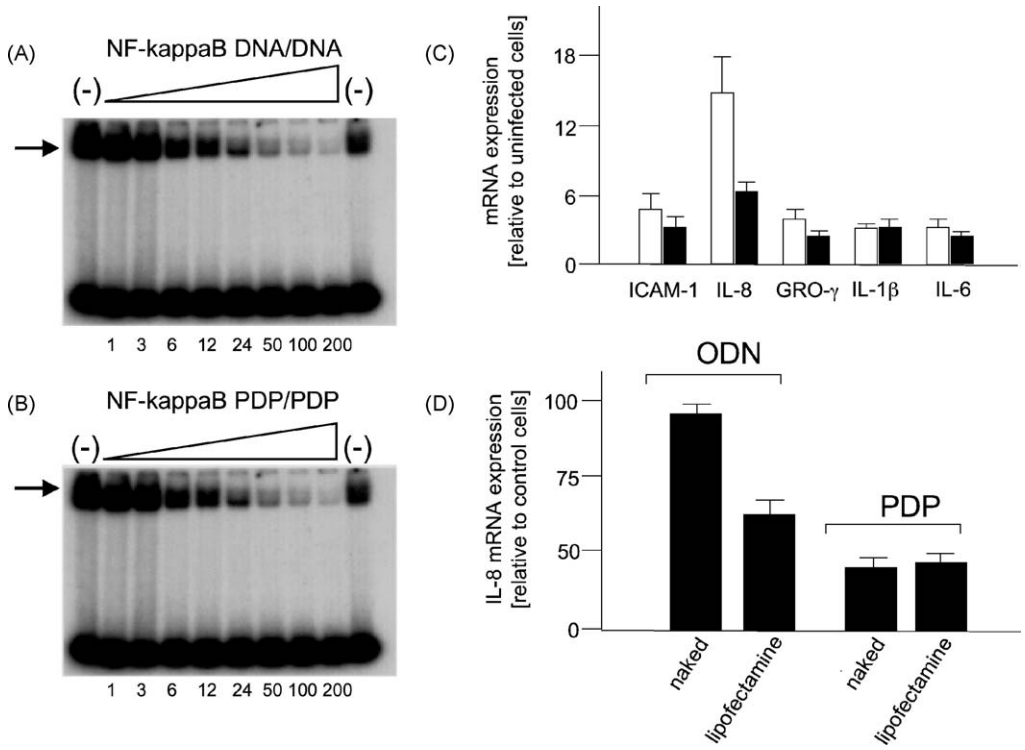

Fig. 5. Electrophoretic mobility shift assay (EMSA) demonstrating decoy activity of the PDP/PDP chimeras. Double-stranded synthetic oligodeoxynucleotides mimicking the NF-κappaB binding sites were labelled with γ^{32}-P-ATP. The binding of the ^{32}P-labelled NF-kappaB target oligonucleotides to purified NF-κappaB p50 dimer protein was performed in the presence of the indicated amounts of competitor double-stranded NF-kappaB ODNs (A) or NF-kappaB PDP/PDP chimeras (B). After binding reactions, the samples were run at constant voltage under low ionic strength conditions on 6% polyacrylamide gels. Gels were dried and subjected to standard autoradiographic procedures (adapted from Romanelli et al., 2001) [75]. Arrows: NF-kappaB/DNA complexes. (C) Effects of scrambled ODNs (white boxes) and PDP-based decoys (black boxes) on accumulation of ICAM-1, IL-8, GRO-γ, IL-1β and IL-6 mRNAs. (D) Comparison of the effects of ODN and PDP-based decoys in the presence or absence of complexation to lipofectamine (protocol of treatment is described in Fig. 3). Values are mean ± SD of three separate experiments.

eukaryotic cells can occur through a nucleic acid channel [67], the complexation with lipofectamine (or other delivery systems) is required *in vitro*, since the degradation of ODNs exposed to foetal calf serum is an important drawback of these transfection approaches [68,69]. On the contrary, we have elsewhere reported that, unlike ODN-based decoys, PDP/PDP chimeras are fully resistant to serum and cytoplasmic extracts [79–83]. This information is of great impact, in our opinion, for the development of stable molecules to be used in non-viral gene therapy [84].

8. Conclusions

The transcription factor decoy (TFD) strategy targeting NF-kappaB appears to be of interest to develop anti-inflammatory approaches for cystic fibrosis. In this field of investigation, the analysis of DNA analogues exhibiting improved activity might be of great interest. In this respect PNA–DNA–PNA chimeras are useful molecules for several points of view: (a) unlike PNAs, they can be complexed with liposomes and microspheres; (b) unlike ODNs, they are resistant to DNAses, serum and cytoplasmic extracts; (c) unlike PNA/PNA and PNA/DNA hybrids, they are potent decoy molecules. We have demonstrated that PDP/PDP chimeras targeting NF-kappaB are strong inhibitors of IL-8 gene expression even in the absence of protection with lipofectamine. Interestingly, unlike ODN-based decoys, PDP/PDP chimeras ere fully resistant to serum and cytoplasmic extracts. This information is of great impact, in our opinion, for the development of stable molecules to be used in non-viral gene therapy. In this respect, we like to underline that IL-8 is one of the master genes in pro-inflammatory processes affecting cystic fibrosis. Accordingly, inhibition of its functions might have a clinical relevance. Our data do not explain the differential effects of NF-kappaB decoys on NF-kappaB regulated genes. It should be underlined, however, that the promoter of most of the genes involved in *P. aeruginosa* mediated inflammation contains sites recognized by other transcription factors. Therefore, further experiments are necessary, in order to clarify the effects of decoy molecules on the NF-kappaB intracellular dynamics, including the determination of the possible effects on the recruitment of NF-kappaB to the promoters of genes which are down-modulated during the decoy treatment (in our case the IL-8 gene). In order to obtain this information, chromatin immunoprecipitation (ChIP) should be considered, despite the fact that this technology has not been extensively employed so far in studying the effects of decoy molecules of the oscillation of transcription factor dynamics. This indeed is a crucial parameters in the way of control gene expression by transcription factors [85,86]. In this respect, in a recent paper by Xu et al. [87]. ChIP studies revealed that the treatment of K562 cells with a decoy oligonucleotide targeting Oct-1 significantly reduced the level of the endogenous gamma-globin gene promoter region DNA (the putative target gene sequences) co-precipitated with the Oct-1 transcription factor [87]. Similar experimental strategy is expected to help in understanding the effects of decoy oligonucleotides targeting NF-kappaB on the transcription of genes carrying NF-kappaB binding sites within their promoter sequences.

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