Biochemical Pharmacology 80 (2010) 1887–1894

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

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*

Roberto Gambari [a,b,*], Monica Borgatti [a], Valentino Bezzerri [c], Elena Nicolis [c], Ilaria Lampronti [a], Maria Cristina Dechecchi [c], Irene Mancini [a], Anna Tamanini [c], Giulio Cabrini [c]

[a] *ER-GenTech and BioPharmaNet, Department of Biochemistry and Molecular Biology, University of Ferrara, Italy*
[b] *Interdisciplinary Center for the Study of Inflammation, University of Ferrara, Italy*
[c] *Laboratory of Molecular Pathology, Laboratory of Clinical Chemistry and Haematology, University-Hospital, Verona, Italy*

ARTICLE INFO

Article history:
Received 2 May 2010
Accepted 28 June 2010

Keywords:
NF-kappaB
Transcription factor decoy
Inflammation
Peptide nucleic acids
PNA–DNA chimeras

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.

* Corresponding author at: Department of Biochemistry and Molecular Biology, University of Ferrara, Via Fossato di Mortara n.74, 44100 Ferrara, Italy.
Tel.: +39 532 424443; fax: +39 532 424500.
E-mail address: gam@unife.it (R. Gambari).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.06.047

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,

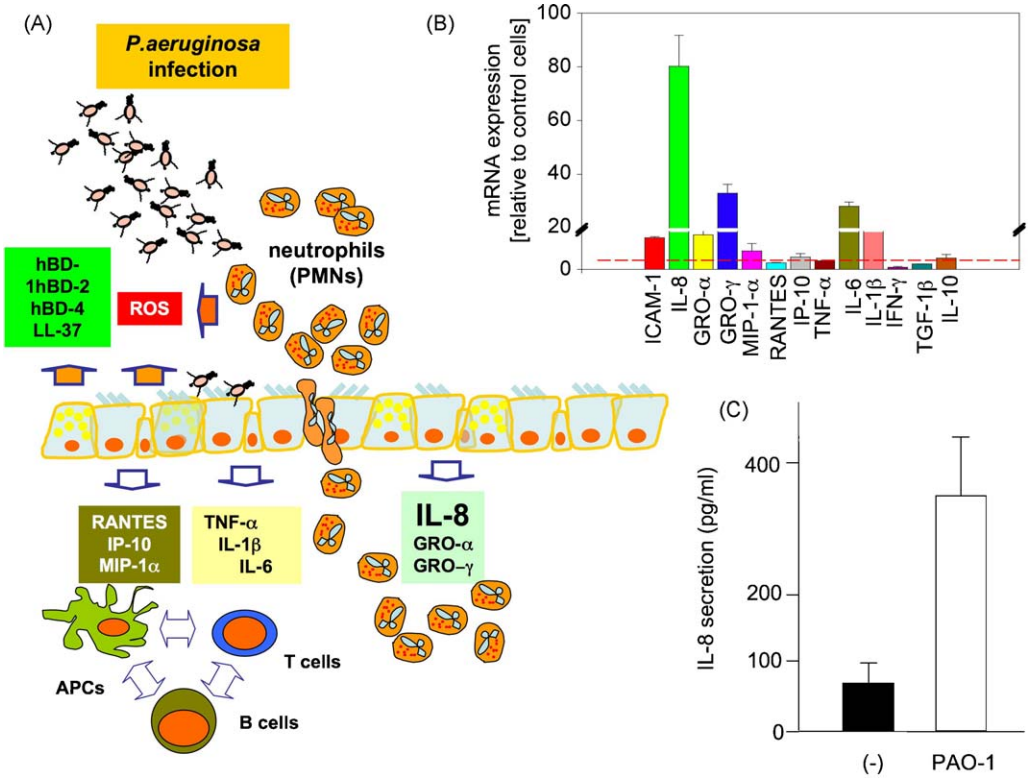

Fig. 1. (A) Early phases of cystic fibrosis lung infection and inflammation. The inflammatory hallmark of this and the following phases is an elevated secretion of the chemokine IL-8, which drives an abnormally elevated recruitment of polymorphonuclear neutrophils in the bronchial walls and lumina. Reduced 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.