



# Identification and analysis of the immune effects of CpG motifs that protect Japanese flounder (*Paralichthys olivaceus*) against bacterial infection

Chun-sheng Liu<sup>a,b</sup>, Yun Sun<sup>a,b</sup>, Yong-hua Hu<sup>a</sup>, Li Sun<sup>a,\*</sup>

<sup>a</sup> Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, PR China

<sup>b</sup> Graduate University of the Chinese Academy of Sciences, Beijing 100049, PR China

## ARTICLE INFO

### Article history:

Received 9 February 2010

Received in revised form

9 April 2010

Accepted 17 April 2010

Available online 24 April 2010

### Keywords:

CpG ODN

*Paralichthys olivaceus*

Immunostimulant

DNA vaccine

## ABSTRACT

CpG-containing oligodeoxynucleotides (ODNs) are known to be immunostimulatory in vertebrate systems and can activate both innate and adaptive immune responses. In this report, we described the selection, identification, and analysis of CpG motifs with immunoprotective effects in Japanese flounder. Sixteen CpG ODNs were synthesized and examined for the ability to inhibit bacterial dissemination in Japanese flounder blood. Four ODNs with the strongest inhibitory effects were selected and mixed to form ODNs 4M. In addition, a plasmid, pCN6, was constructed that contains the sequences of the four selected ODNs. When administered into Japanese flounder via intraperitoneal injection, both ODNs 4M and pCN6 could, in dose and time dependent manners, afford short-term protection against the infections of two different bacterial pathogens. Immunological analyses showed that ODNs 4M and, especially, pCN6 activated head kidney macrophages and enhanced serum bactericidal activity via probably the alternative pathway of complement activation. When used as a DNA vaccine to immunize Japanese flounder, pCN6 conferred apparent protections (42.9% and 52.6%, respectively, in terms of relative percent survival) against the challenges of two different fish pathogens at 4-week post-vaccination. Transcriptional analysis showed that vaccination with pCN6 upregulated the expression of the genes encoding NKEF, MHC II $\alpha$ , IL-1 $\beta$ , Mx, and MHC I $\alpha$ . These results demonstrate that ODNs 4M and pCN6 are immunostimulatory in Japanese flounder and can induce short- and long-term nonspecific protections against bacterial infections.

© 2010 Elsevier Ltd. All rights reserved.

## 1. Introduction

Pathogen-associated molecular patterns (PAMPs) are molecular or structural features that are associated commonly with microbial pathogens but are generally lacking in vertebrate systems. PAMPs are recognized by a group of molecules of vertebrate immune systems called pattern recognition receptors (PRRs), which play a crucial role in innate immune defense by binding to PAMPs on invading pathogens and initiating immune responses that facilitate pathogen clearance. Among the PRRs that have been identified to date is the toll-like receptor (TLR) 9, a signaling molecule which in mammals is found to be expressed predominantly in B cells and dendritic cells. The ligand of TLR9 is unmethylated cytosine-phosphate-guanine (CpG) dinucleotides, which are qualified as PAMPs by the fact that in vertebrate genomes CpG motifs are suppressed and methylated, whereas in bacterial and viral genomes CpG motifs are prevalent, unmethylated, and flanked by sequences that are

different from those in vertebrate DNA [1–3]. Interaction between TLR9 and CpG DNA induces immune responses by activating the nuclear factor NF- $\kappa$ B through the MYD88-dependent pathway [4–6]. Recent studies have demonstrated that synthetic oligodeoxynucleotides (ODNs) containing CpG motifs (CpG ODNs) can mimic bacterial CpG dinucleotides and produce various immune effects [3]. Based on their structural differences, CpG ODNs can be classified into three major groups named A, B, and C [7,8]. B-class ODNs are constructed on phosphorothioate (PS) backbones, in which a non-bridging oxygen on the phosphate linkage is replaced by a sulfur so that the DNA is resistant against degradation by nucleases. B-class ODNs are usually 20–30 nucleotides in length and contain one or more CpG motifs with various flanking sequences. A-class ODNs contain CpG motifs on phosphodiester (PO) backbones with PS-polyguanosines at the 5' or 3' end. C-class ODNs contain multiple CpG motifs on PS backbones and the TCG motif at the 5' end followed by a palindrome sequence. The three classes of CpG ODNs differ in immune properties [7,9,10]. B-class ODNs are known to activate B cells and dendritic cells, whereas A-class ODNs are poor B cell activators but are potent inducers of IFN- $\alpha$ . Owing to their immunopotentiating capacities, CpG ODNs

\* Corresponding author. Tel./fax: +86 532 82898829.

E-mail address: [lsun@ms.qdio.ac.cn](mailto:lsun@ms.qdio.ac.cn) (L. Sun).

have been studied extensively as vaccine adjuvants, and some CpG ODNs have been found to enhance specific immune responses induced by antigens [8].

CpG with immune effects have been identified for a number of fish species, including rainbow trout (*Oncorhynchus mykiss*) [11–15], Atlantic salmon (*Salmo salar* L.) [16], common carp (*Cyprinus carpio* L.) [17–20], grass carp (*Ctenopharyngodon idellus*) [21], chinook salmon (*Oncorhynchus tshawytscha*) [22], and catfish (*Ictalurus punctatus*) [23]. Although recent studies showed that an Atlantic salmon-effective CpG ODN was also immunostimulatory in Japanese flounder (*Paralichthys olivaceus*) [24,25], no extensive selection of CpG motifs has been conducted in Japanese flounder, and no plasmid form of CpG motifs has been tested in this fish species either. Since Japanese flounder is an economically valued fish species and one of the principle fish species cultured in China, we in this study aimed to identify CpG motifs that, in the form of ODN and plasmid DNA, can stimulate the immune system of Japanese flounder and confer protection against bacterial infection.

## 2. Materials and methods

### 2.1. Bacterial strains and growth conditions

*Aeromonas hydrophila* AH1 and *Edwardsiella tarda* TX1 are fish pathogens that have been reported previously [26,27]. *Escherichia coli* DH5 $\alpha$  was purchased from Takara (Dalian, China). All strains were cultured in Luria-Bertani broth (LB) medium [28] at 37 °C (for *E. coli*) or 28 °C (for *A. hydrophila* and *E. tarda*). Appropriate antibiotics were supplemented at the following concentrations: ampicillin, 100  $\mu$ g/ml; kanamycin, 50  $\mu$ g/ml; chloramphenicol, 25  $\mu$ g/ml.

### 2.2. CpG ODNs

Sixteen CpG ODNs were used in this study, of which, ten have been reported previously: 1668 [14], A [17], B [17], 1670 [12,16], 1677 [16], 1679 [16], 1826 [21,22], 2006 [21], 2133 [12], and 2143 [12]. The immunological properties of these CpG ODNs are summarized in the review by Carrington and Secombes [7]. ODNs 201, 203, 205, 207, 208, and 209 (Table 1) were designed specifically for this study. All the CpG ODNs were constructed on phosphorothioate backbones and synthesized by Sangon (Shanghai, China). The synthesized ODNs were solubilized in sterile deionized water and stored at –20 °C.

### 2.3. Fish

Japanese flounder (*P. olivaceus*) were purchased from a local fish farm and acclimatized in the laboratory for two weeks before experimental manipulation. Fish were fed daily with commercial dry pellets and maintained at ~22 °C in aerated seawater that was changed twice daily. Before experiments, fish were randomly sampled for the examination of bacterial recovery from blood, liver,

kidney, and spleen. Fish were used for experiment only when no bacteria could be detected from any of the examined tissues of the sample fish. Fish were anaesthetized with tricaine methanesulfonate (Sigma, St. Louis, MO, USA) prior to experiments involving injection, blood collection, or sacrifice. Sacrifice was performed by administration into the fish an overdose of tricaine methanesulfonate as described previously [26].

### 2.4. Bacterial recovery from fish blood

Bacterial recovery from fish blood was performed as described previously [27]. Briefly, fish were sacrificed as described above, and blood was taken from the caudal veins under aseptic conditions. The blood was plated on LB agar plates supplemented with ampicillin and chloramphenicol, respectively, for the selection of AH1 and TX1. The plates were incubated at 28 °C for 48 h, and the colonies that emerged on the plates were enumerated. The genetic natures of the colonies were verified by PCR using primers specific to AH1 and TX1 and sequence analysis of randomly selected PCR products.

### 2.5. Effect of CpG ODNs on bacterial dissemination in blood

Japanese flounder (~8 g) were divided randomly into 17 groups (5 fish/group) and administered intraperitoneally (i.p.) with each of the 16 CpG ODNs at 0.1  $\mu$ g/fish or phosphate buffered saline (PBS) as a control. At 24 h post-administration, the fish were challenged with AH1. Bacterial recovery from blood was determined at 12 h post-challenge as described above.

### 2.6. Effect of CpG ODNs 4M in relation to dose and time

To examine the effect of dose on the antibacterial property of ODNs 4M, Japanese flounder were divided randomly into four groups (5 fish/group) and administered i.p. with PBS (control) or ODNs 4M at the concentrations of 0.016  $\mu$ g, 0.08  $\mu$ g, and 0.4  $\mu$ g per fish, respectively. The fish were challenged with AH1 and examined for bacterial recovery as above. To examine the effect of time on the antibacterial property of ODNs 4M, Japanese flounder were divided randomly into six groups; three groups (A, B, and C) were injected i.p. with PBS and the other three groups (D, E, and F) were injected i.p. with ODNs 4M at 0.4  $\mu$ g/fish. Groups A and D, B and E, and C and F were challenged with AH1 at 12 h, 24 h, and 48 h post-ODN administration, respectively. Bacterial recovery from blood was determined as described above. The effect of dose and time on the anti-*E. tarda* ability of ODNs 4M was determined in the same fashion.

### 2.7. Construction of pCN6

pCN6 was constructed by inserting linker 43F (5'- GATCGCGC GCGCGCTCTATTCGTCGTTGGTTGTCGTTTTGGTG -3') into plasmid pCN3 [29] at the BamHI site; the recombinant plasmid was digested with BamHI and ligated with linker 40F (5'- GATCACGTACGTACGT CTATGATCTCGCTCGCTCGCTATG -3').

### 2.8. Plasmid preparation

*E. coli* DH5 $\alpha$  harboring pCN3 and pCN6 were cultured in LB medium, and endotoxin-free plasmids were extracted using EndoFree plasmid Kit (Tiangen, Beijing, China). The purity of the purified plasmid DNA was analyzed spectrophotometrically by measuring absorbance at  $A_{260/280}$  and  $A_{260/230}$ . The integrity of the plasmid DNA was assessed by agarose gel electrophoresis.

**Table 1**  
Sequences of the CpG ODNs designed for this study.

| ODN | Sequences (5' → 3') <sup>a</sup> |
|-----|----------------------------------|
| 201 | GATCACGTACGTACGTCTAT             |
| 203 | GATCTCGCTCGCTCGCTAT              |
| 205 | GATCGCGTGGCTGCGTCTAT             |
| 207 | GATCGCGCGCTGCGTCTAT              |
| 208 | GATCGCGCGACGCGCTCTA              |
| 209 | GATCGCGCGCGCGCTCTAT              |

<sup>a</sup> CpG motifs are underlined.

Download English Version:

<https://daneshyari.com/en/article/2432864>

Download Persian Version:

<https://daneshyari.com/article/2432864>

[Daneshyari.com](https://daneshyari.com)