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Short communication

Immunostimulatory oligodeoxynucleotides induce dolphin neutrophil NADPH-oxidase activation in a CpG-independent but phosphorothioate backbone-dependent manner

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Abstract

Immunocytes, which include antigen-presenting cells, B cells, natural killer cells and neutrophils, can be stimulated directly or indirectly with bacterial DNA and synthetic oligodeoxynucleotides (ODNs) with different structures and sequences. In the present study, we investigated the effect of synthetic ODNs on the respiratory burst of dolphin neutrophils using a chemiluminescence assay. Phosphorothioate (PS)-ODNs dose-dependently induced the respiratory burst, while phosphodiester (PO)-ODNs did not, regardless of CpG-content. The PS-ODN-induced activity was completely abolished by the flavoprotein inhibitor diphenyleneiodonium, which indicates that the NADPH-oxidase is activated by PS-ODNs. These results reveal that PS-ODNs induce dolphin neutrophil NADPH-oxidase activation in a CpG motif-independent but phosphorothioate-dependent manner.

Keywords: Dolphin; Neutrophils; NADPH-oxidase; Oligodeoxynucleotides; Phosphorothioate backbone

1. Introduction

DNA has direct immunostimulatory effects aside from its function in encoding the genetic material. The bacterial deoxyribonucleic acid fraction from *Mycobacterium bovis* activates natural killer (NK) cells and induces interferon (IFN) production leading to tumor suppression in some murine models, whereas vertebrate DNA lacks this activity [1–3]. In addition, bacterial DNA, but not vertebrate DNA, stimulates murine B-cell proliferation and immunoglobulin secretion [4]. It has been demonstrated that the structural requirement for this effect is the presence of CpG dinucleotides in particular base contexts, which are termed 'CpG motifs' [5]. In mice and humans, the bacterial DNA and synthetic oligodeoxynucleotides (ODNs) effectively trigger B cells to upregulate the expression of costimulatory molecules and MHC

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class II, and to secrete IL-6, IL-10, and immunoglobulin [5–9]. CpG DNA can also directly activate antigen-presenting cells (APCs), including monocytes, macrophages and dendritic cells, to produce IL-12, IL-6, TNF, and IFN [10–12] and to express increased levels of costimulatory molecules, which are required for the indirect activation of NK cells, thereby enhancing lytic activity and IFN secretion [13,14].

In contrast to native phosphodiester backbone ODNs (PO-ODNs), phosphorothioate-modified ODNs (PS-ODNs) have sequence-independent biological activities [15]. PS-ODNs, but not PO-ODNs, induce the activation of macrophages and B cells [16,17]. Recently, Bylund and colleagues discovered that human neutrophil respiratory burst and degranulation were increased by PS-ODNs regardless of CpG content, conversely, the same PS-ODNs inhibited neutrophil chemotaxis and phagocytosis [18]. This impact of PS-ODNs on neutrophil function is unique and distinct from that exerted on other immune cells, with respect to both the identity of the activating DNA molecules and the regulation of the effector functions.

We have previously demonstrated that although dolphin neutrophils respond to several soluble and particulate stimuli, similarly to human neutrophils, they are refractory or weakly responsive to bacterial agents [19]. Although the mechanism underlying this difference is unknown, the fundamental components and structures for NADPH-oxidase activity in dolphin neutrophils are basically similar to those in other mammals [20–23]. The purpose of the present study was to investigate whether PS-ODNs activate directly dolphin neutrophil respiratory burst activity in vitro. We selected several ODNs with motifs that are optimal for the activation of human neutrophils to investigate species differences.

2. Materials and methods

2.1. Chemicals and oligodeoxynucleotides

Luminol and diphenyleneiodonium chloride (DPI) were purchased from Wako Pure Chemical (Tokyo, Japan) and phorbol 12-myristate 13-acetate (PMA) from Sigma (St Louis, MO). Eagle's MEM was from Nissui (Tokyo, Japan). HEPES was from Dojindo (Kumamoto, Japan) and LYMPHOPREP from Nycomed Pharma (Oslo, Norway). Nuclease-resistant phosphorothioate ODNs (PS-ODNs) were obtained from SGSDNA (Stockholm, Sweden). Phosphodiester ODNs (PO-ODNs) were purchased from GENSET KK (Kyoto, Japan). The ODNs sequences are listed in Table 1. Calf thymus (CT) DNA was from Sigma. PS- and PO-ODNs were diluted in pure water to 1 mM and stored at −20 °C until use. Further dilutions were made in HBSS and used in vitro at final concentrations of 0.15625, 0.3125, 0.625, 1.25, 2.5 and 5 μM.

Table 1
Oligodeoxynucleotide sequences

Designations	DNA sequences (5′-3′)[a]	ROS production[b]
Phosphorothioates		
1600	**TCG TCG** TTT TGT **CG**T TTT GT**C G**TT	+
1700	**TGC TGC** TTT TGT **GC**T TTT GT**G C**TT	+
Phosphodiesters		
2199	**TCG TCG** TTT TGT **CG**T TTT GT**C G**TT	−
2299	**TGC TGC** TTT TGT **GC**T TTT GT**G C**TT	−

[a] The prototype CpG-ODNs sequences 1600 and 2199 are derived from ODN 2006 [9]. The non-CpG-ODNs 1700 and 2299 were synthesized by replacing the CpG dinucleotide in ODNs 1600 and 2199 with a GpC dinucleotide.

[b] Measured as neutrophil release of reactive oxygen species in response to 5 μM of the ODNs. The peak values were approximately 3.0 Mcpm for activating ODNs (+), while ODNs (−) gave no significant levels of induction relative to the background level.

2.2. Animals

Bottlenose dolphins (*Tursiops truncatus*, bodyweight of 240–350 kg; >5 years of age) were used as the source of neutrophils. They were maintained for the purpose of exhibition and education at the Shinagawa Aquarium, Shinagawa-ku, Katsushima, Tokyo, Japan and were periodically checked for health by aquarium staff.

2.3. Preparation of dolphin neutrophils

Peripheral blood samples (~10 ml/individual; $n=4$) were drawn from the ventral tail fluke into heparinized vacutainers and kept cool until analysis. All samples were examined within 6 h after blood