Available online at www.sciencedirect.com
ScienceDirect
Veterinary Immunology and Immunopathology 115 (2007) 24–34
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Innate immune responses induced by classes of CpG oligodeoxynucleotides in ovine lymph node and blood mononuclear cells

Jayaum S. Booth [a], Anil K. Nichani [a,1], Ponn Benjamin [a], Arshud Dar [a], Arthur M. Krieg [b], Lorne A. Babiuk [a], George K. Mutwiri [a,*]

[a] *Vaccine & Infectious Disease Organization, University of Saskatchewan, 120 Veterinary Road, Saskatoon, Sask., Canada S7N 5E3*
[b] *Coley Pharmaceutical Group, Wellesley, MA, USA*

Received 1 May 2006; received in revised form 25 August 2006; accepted 21 September 2006

Abstract

CpG ODN signal through Toll-like receptor 9 (TLR9) and trigger a cascade of events that lead to activation of innate and adaptive immune responses. Our current understanding of the immunobiology of host responses to CpG is based largely on studies on peripheral blood mononuclear cells (PBMC) and splenocytes. Little is known regarding CpG-induced responses in other lymphoid tissues. In the present study, we investigated responses induced by CpG in both PBMC and lymph nodes. Cells were isolated from the superficial cervical lymph node (LNC) and blood and then stimulated with CpG ODN (either A-, or B- or C-class ODN). Cytokine production was assayed by ELISA, and lymphocyte proliferation was determined by ^{3}H-thymidine incorporation. NK-like cytotoxicity was analyzed by lysis of ^{51}Cr-labelled target cells. All three classes of CpG induced IFNα and IFNγ in LNC. In contrast, only A and C-class ODN induced IFNα and IFNγ in PBMC. Moreover, the IFN levels in LNC were 20–40-fold higher than in PBMC. Furthermore, all classes of ODN induced higher IL-12 levels in LNC (five- to six-fold) than in PBMC. Both B and C-class ODN induced good proliferative responses in PBMC and LNC, but the A-class ODN did not induce proliferation of PBMC and only induced moderate proliferation of LNC. A-class ODN induced significant NK-like activity in LNC. Thus, all three classes of CpG ODN induced similar responses in LNC, and these responses were consistently higher than in PBMC. These observations indicate that CpG ODN-induced responses differ between blood and lymph nodes, and suggest that the functional classification of CpG ODN based on PBMC responses may not be directly applicable to cells from other immune tissues.

Keywords: CpG oligodeoxynucleotides; Innate immune responses; Sheep

1. Introduction

The innate immune system has a conserved network of receptors called pathogen recognition receptors (PRRs) that have the ability to recognize pathogen associated molecular patterns (PAMPs) as 'danger' signals (Dempsey et al., 1996). On recognition of their respective ligands, PRRs are capable of inducing a variety of immune responses. Toll-like receptors

* Corresponding author. Tel.: +1 306 966 1511; fax: +1 306 966 7478.
E-mail address: george.mutwiri@usask.ca (G.K. Mutwiri).
[1] Current address: Canadian Food Inspection Agency, Moose Jaw, Sask., Canada.

0165-2427/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2006.09.008

(TLRs) constitute one family of PRR. At least eleven TLRs have been identified in mammals and they provide a sensory mechanism for the detection of infectious threats (O'Neill, 2004; Takeda et al., 2003). For example, TLR3, TLR4, TLR7/8 and TLR9 recognize double-stranded RNA, lipopolysaccharides (LPS), single stranded RNA/imidazoquinolines and CpG or bacterial DNA respectively (Alexopoulou et al., 2001; Hemmi et al., 2000; Jurk et al., 2002; Medzhitov et al., 1997; Poltorak et al., 1998).

CpG dinucleotides are present at the expected frequency (1/16 base pair) in bacterial DNA. In contrast, the frequency of CpG is suppressed to about one quarter of the predicted value in vertebrate DNA and also the cytosine is often methylated (Krieg and Wagner, 2000). Based on these differences, it has been proposed that vertebrates have evolved to recognize CpG DNA as a mechanism of detecting the presence of pathogens and stimulating the innate immune system. Bacterial DNA, but not vertebrate DNA can stimulate mammalian immune cells (Krieg et al., 1995). Synthetic oligonucleotides containing CpG motifs mimic the immunostimulatory effects of bacterial DNA (Krieg et al., 1995).

Both in vitro and in vivo studies have demonstrated that CpG ODN are potent activators of the innate immune system in numerous species including humans, non-human primates, mice, cattle, sheep, pigs, horses, dogs, cats, chickens and fish (Brown et al., 1998; Jorgensen et al., 2001; Kamstrup et al., 2001; Pontarollo et al., 2002; Rankin et al., 2001; Verthelyi and Klinman, 2003; Wernette et al., 2002; Zhang et al., 2001). CpG ODN have been shown to be protective against a variety of pathogens including bacteria, viruses and protozoa in numerous animal models (Ayash-Rashkovsky et al., 2005; Gomis et al., 2003; Gramzinski et al., 2001; Harandi et al., 2003; Shi et al., 2005; Tewary et al., 2004; Wedlock et al., 2005). When given with an antigen, CpG has the ability to enhance antigen specific immune responses and this has been shown in humans and numerous animal species (Farkas et al., 2004; Hartmann et al., 2005; Ioannou et al., 2002; Klinman et al., 1999; Moss et al., 2000; Rothenfusser et al., 2004).

Numerous in vitro studies have revealed that in mice, a variety of cell types are activated directly or indirectly by CpG ODN. These include B lymphocytes, monocytes, macrophages, dendritic cells, NK cells and even mast cells and, depending on the cell type activated they proliferate, upregulate MHC class I and II, B7-1 and B7-2 co-stimulatory molecules, or express a broad range of cytokines including IL-1, IL-6, IL-10, IL-12, IFN-α, IFN-γ and TNF-α (Klinman et al., 2002; Krieg, 2002; Krug et al., 2001; Sun et al., 1998; Zhu and Marshall, 2001). In humans, plasmacytoid dendritic cells (pDCs) and B cells express TLR-9 and are directly stimulated by CpG ODN to proliferate, produce cytokines and upregulate co-stimulatory molecules (Bauer et al., 2001; Hornung et al., 2002; Vollmer et al., 2004). There have been relatively few studies in ruminants, but those that have been reported indicate that CpG ODN induce IFN-α and IFN-γ secretion in bovine PBMC in vitro, but no NK-like cytotoxicity (Mena et al., 2003a,b). It has also been reported that CpG ODN induced IL-6, IL-12 and IFN-γ in bovine PBMC whereas B lymphocyte produced only IL-6 (Zhang et al., 2001). However, CpG ODN stimulation of ovine PBMC induced IFNα and NK-like cytotoxicity but no IFNγ secretion (Mena et al., 2003a,b), whereas stimulation of ovine lymph node cells induced both IFNα as well as IFNγ (Nichani et al., 2004a). However, in these studies, the capacity of CpG to induce IL-12, a cytokine that plays a key role in the development of Th-1type immune response, was not evaluated (Mena et al., 2003a,b).

Three distinct classes of CpG oligodeoxynucleotides (ODN) have been characterized: (i) A-class ODN (phosphodiester/phosphorothioate backbone with a poly G tail) trigger maturation of antigen-presenting cells (APCs) and directly induce the secretion of high levels of IFNα from pDCs but little or no B cell activation (Ballas et al., 1996; Krug et al., 2001; Verthelyi et al., 2001), (ii) B-class ODN (multiple CpG motifs on a phosphorothioate backbone) trigger B cells to proliferate and induce little or no IFNα (Gursel et al., 2002; Hartmann et al., 2000; Vollmer et al., 2004), and (iii) C-class ODN (Hexameric CpG motif [5′-TCGTCGTT-3′] linked by a T spacer to GC-rich palindromic sequences) have the functional properties of both A and B class ODN (Jurk et al., 2004; Vollmer et al., 2004). Most of these observations were made using human PBMCs or mouse splenocytes. Little is known about the immunostimulatory effects of these ODN in other lymphoid tissues. A recent study indicated that the biodistribution of CpG is more dynamic than previously thought (Noll et al., 2005). These investigators showed that following subcutaneous injection of CpG ODN, approximately 10–15% of the ODN were present in lymph node and organs after 4 h whereas in serum, the level of the ODN was very low (Noll et al., 2005). They also observed a significant increase in ODN concentration between 4 and 24 h in the lymph nodes. The accumulation of ODN in lymph nodes suggests