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Research paper

CpG oligodeoxynucleotide protect neonatal piglets from challenge with the enterotoxigenic *E. coli*

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ABSTRACT

CpG motifs activates mammalian lymphocytes and macrophages to produce cytokines and polyclonal Ig. These include IFN-γ, IL-12, TNF-a, which are important in the control of bacterial infection. But thus far, the innate immunostimulatory effects of CpG ODN against pathogen have been established mainly in mouse, monkey, sheep, chicken, but not in neonatal piglets. The purpose of this study is to determine the potential protection of CpG ODN against enterotoxigenic *Escherichia coli* (ETEC) (with which neonatal piglets were susceptible to infection in our lab) in neonatal piglets. Here, we show intranasal (IN)—mucosal and intramuscularly (IM) systemic administration of CpG ODN could enhance innate cellular (cytokine) immunity in the sera and intestine mucosa post challenge, and thereafter the development of antigen-specific antibodies in piglets. IN and IM immunizations of neonatal piglets without antigen both reduced the ETEC excretion and alleviated diarrhoea symptoms upon challenge, and IN route had better protection effects than IM route. Protection in this study was linked to induction of a Th1 response which induced by CpG ODN. Co-delivery with Emulsigen (EM), could improve protection mediated by CpG ODN. These observations indicate that IN administration of 100 μg/kg CpG ODN with 20% EM codelivery may represent a valuable strategy for induction of innate immunity against ETEC infection in neonatal piglets.

1. Introduction

Diarrhoea remains an important causal agent of morbidity and mortality in livestock and is one of the most common diseases of all ages of piglets worldwide (Toledo et al., 2012; Wang et al., 2013a). Diarrheagenic *Escherichia coli* (DEC) is a common cause of diarrhea, including enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAEC). Enterotoxigenic *E. coli* (ETEC) causes neonatal and post weaning diarrhea (PWD) in pigs, resulting in high economic losses in many swine producers (Vu-Khac et al., 2007). Diarrhea can negatively affect food consumption and production efficiency (Madoroba et al., 2009), and may

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increase other disease, such as bacterial overgrowth, terminal septicaemia (Lee et al., 2008) and mortality (Hur and Lee, 2012; Zhang et al., 2007a).

Although ETEC can control by antibiotics, it's often too late for piglets with visible clinical signs, as toxin has been produced in the gut and systemic spread throughout the body (Thi et al., 2012). Nevertheless, the continuous use and misuse of antibiotics have led to the emersion of the antibiotic-residue in the poultry product. And antibiotic resistance now has become a great concern (Looft et al., 2012). Passive transfer of cholesterol or lactogenic immunity developed by vaccination of the pregnant sow may be another choice to prevent the Neonatal infections. However, the passive protection decreases with age, and lactogenic immunity disappears at weaning. Therefore, piglets will become highly susceptible to enteropathogens such as ETEC. So far, there are no effective oral vaccines available against ETEC infections (Hur and Lee, 2012).

Multicellular organisms have a number of defense systems which enable them to resist infection by pathogens. One of these, the innate immune system, is a highly conserved system that has been described in species as diverse as insects and humans (O'Neill et al., 2013). In this study, we have considered the possibility that activation of the innate immune system can provide protection against ETEC infection. Activation of the innate immune system is a consequence of the recognition of pathogen-specific molecules, and it then provides protection in a non-specific manner. The Toll-like receptors (TLRs) on host cells have been shown to play a key role in the recognition of these pathogen-specific molecules. To date 10 TLRs have been identified in humans and mice, and TLR9 has been shown to recognize unmethylated cytosine and guanosine dinucleotides (CpG) and their flanking regions of DNA (Kim et al., 2012). Such CpG motifs are frequently abundant in the DNA of pathogens, but suppressed in eukaryotes (Sharma and Fitzgerald, 2011).

DNA containing CpG motifs (CpG DNA) triggers humoral immunity by inducing B cell and plasmacytoid dendritic cells to proliferate and secreting a varity of cytokines, chemokines and IgM (Okuda et al., 2014; Wang et al., 2013b). CpG DNA also directly activates monocytes and macrophages to secrete cytokines, especially IL-12, TNF-α and IFN-αβ (Grassia et al., 2013; Xu and Banchereau, 2014). In contrast, CpG DNA fails to directly stimulate isolated NK cells. However, CpG DNA stimulate macrophages which can produce the cytokines to act on NK cells to induce lytic activity and IFN-γ secretion (Souza-Fonseca-Guimaraes et al., 2012). The IFN-γ that is secreted in response to CpG DNA promotes B cell activation and Ig secretion. In addition to murine cells, human B cells, monocytes, and NK cells are strongly activated by CpG DNA, although the optimal flanking bases and the spacing of the CpG motifs are slightly dissimilar.

Neonates have an increased susceptibility to infection due to inherent limitations of their immune system. It is believed that neonates exhibit functionally impaired antigen presentation, shorter lived and weaker antibody responses, a Th2-type immune response bias and a decreased overall cell-mediated immune response if compared with adults (Demirjian and Levy, 2009). The intestinal compartment in neonates is almost devoid of immune cells and this could contribute to the high sensitivity observed in response to intestinal pathogens (Lacroix-Lamandé et al., 2009). It is therefore of a particular interest to develop strategies for stimulating their intestinal immune system to improve their resistance to infection. The Gram-negative bacterium ETEC is considered to be one of the main causes of sickness and death in neonatal piglets (Zhang et al., 2007). Yet, little is known about neonatal gut mucosal immune responses induced by CpG when challenged with ETEC. Our previous studies indicate that when presented with an antigen, CpG has the power to enhance antigen specific immune responses and this has been demonstrated in humans and numerous animal species including pigs (Cao et al., 2010, 2011; Linghua et al., 2006; Zhang et al., 2006a). And it was demonstrated in our laboratory that CpG ODN delivered by systemic or mucosal immunization routes could effectively mucosal immune responses in weaned and aged piglets (Cheng et al., 2010a; Ming et al., 2013; Zhang et al., 2007b). Also, we found that neonatal piglets (1–3 days of age) are susceptible to infection with ETEC 196 strain and show severe signs of diarrhoea, weight loss and moderate to mild fever. Therefore, using this model, our research is focused on utilizing innate immune modulators (CpG ODN) to activate and imprint neonatal piglets towards a Th1 type of response, which ultimately will help to enhance neonatal immunity against infectious diseases such as ETEC.

2. Materials and methods

2.1. Animals

Some 1–3-day-old Landrace × Large White pigs (F4-seronegative as determined by ELISA) were used in this study. Five groups of 10 piglets (2–3 kg per piglet) which were divided randomly. All pigs were from the Swine Breeding Center of Guangzhou. F4 antigen was obtained from China Institute of Veterinary Drug Control.

2.2. CpG-ODN and ETEC

CpG ODN (5′-ggTGCATCGATTTATCGATGCAGggggg-3′) was synthesized in the TaKaRa Biotech Co., and the porcine-specific motif, which was used in the present study, was the equivalent of sequence D19 used by Kamstrup et al. (2001), in which phosphodiester nucleotides were shown in upper case and phosphorothioate nucleotides were shown in lower case. CpG ODN was resuspended in phosphate buffer saline (PBS) at a concentration of 2 mg/ml, and stored at −20 °C.

ETEC 196 strain (O8:K88ac) was obtained from China Institute of Veterinary Drug Control. ETEC strain propagated for 24 h at 37 °C in Brain Heart Infusion broth (Oxoid, Unipath, Drongen, Belgium). Bacteria were collected by centrifugation, washed and suspended in PBS to a concentration of 10^{10} CFU/ml.