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Research paper

TLR9 agonists: Immune mechanisms and therapeutic potential in domestic animals

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ABSTRACT

Toll like receptors (TLRs) are transmembrane glycoproteins that recognize conserved microbial molecules. Engagement of TLRs activates innate and adaptive immunity. TLR-mediated activation of immune cells results in upregulation of cytokines, chemokines and costimulatory molecules. These early innate responses control pathogen spread and initiates adaptive immune responses. Synthetic CpG oligodeoxynucleotides (ODN), agonists for TLR9, had shown great promise as immunotherapeutic agents and vaccine adjuvants in laboratory animal models of infectious disease, allergy and cancer. However, it has become apparent that CpG ODN are less potent immune activators in domestic animals and humans. The disparity in immune responses between rodents and mammals has been mainly attributed to differences in cellular expression of TLR9 in the various species. In this article, our current understanding of the immune mechanisms, as well as the potential applications of CpG ODN will be reviewed, with particular emphasis on domestic animals.

1. Introduction

The immune system uses a variety of germ-line encoded receptors to recognize conserved microbial molecules. These receptors, generally referred to as pattern recognition receptors (PRRs) include Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-like receptors (RLRs) and C-lectin type receptors (CLRs) (Creagh and O'Neill, 2006). TLRs are the most widely studied PRRs. The first member of the Toll family of proteins was identified in Drosophila during a screen for embryonic polarity genes (Hashimoto et al., 1988). Subsequently, Medzhitov et al. (1997) identified a human homologue of the Drosophila Toll protein and showed that it was involved in activation of immunity. Since then, at least 13 TLRs have been described in mammals, and activation of the receptors triggers an intracellular signalling cascade that involves recruitment of signal transduction molecules, primarily MyD88 (Medzhitov and Janeway, 2000). While the majority of TLRs utilize the MyD88 pathway, others are dependent on TRIF, and at least in the case of TLR4, both MyD88 and TRIF pathways are activated (Takeda and Akira, 2004). Intracellular activation of TLR results in nuclear translocation of NFkB that induces production of inflammatory cytokines, chemokines, and upregulation of costimulatory molecules in immune cells (Takeda and Akira, 2004). These innate immune responses control pathogen replication, and initiates and directs the development of adaptive immune responses.

TLRs can be divided into two categories; (i) those that are located intracellularly in the endosomal compartment (TLR3, 7, 8 and 9) and recognize microbial nucleic acids, and (ii) those that are located extracellularly (TLR2, 4, 6, 10) and recognize other microbial components including LPS and flagellin. However, there is evidence to suggest that the cellular localization of TLR can vary depending on the tissue examined. For example, TLR4, which is expressed on the cell surface in most cells has been demonstrated intracellularly in intestinal epithelial cells (IEC) (Mueller et al.,

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2006). It is thought that TLR4 is located intracellularly in IEC to prevent unnecessary responses to commensal bacteria in the intestinal lumen. Also, TLR9, which is usually located intracellularly, may be expressed extracellularly in lung tissue (B. Singh, personal communication). The physiological significance of this is unknown but may be regulated in response to cytokines and pathogens (Miettinen et al., 2001; Krutzik et al., 2003).

TLR agonists are potent immune activators and have attracted a great deal of interest due to their potential as immunotherapeutic agents and vaccine adjuvants. This article will focus on the immune mechanisms and potential applications of TLR9 stimulation with its agonist CpG ODN.

2. Cellular activation with CpG ODN

CpG ODN activates cells from humans and a variety of animal species including non-human primates, mice, sheep, pigs, cattle, fish, horses, dogs and cats (reviewed by Mutwiri et al., 2003).

It is thought that only a small subset of cells express TLR9 and are directly activated by CpG ODN stimulation. These include B cells, plasmacytoid and conventional dendritic cells (pDC and cDC), macrophages and monocytes (reviewed by Wilson et al., 2006). However, many more cell populations do not express TLR9 but may be indirectly stimulated by cytokine signals and contribute to the overall response to CpG ODN (Wilson et al., 2006). Depending on the cell populations stimulated with CpG ODN, they show increased proliferation, expression of costimulatory molecules or production of a range of cytokines including IL-1, IL-6, IL-10, IL-12, IFNa, IFNg and TNFa (Klinman et al., 2002; Krieg, 2002; Krug et al., 2001; Sun et al., 1998; Zhu and Marshall, 2001).

Immune responses induced by TLR9 activation can vary remarkably from one species to another. It has been known for a long time that CpG ODN trigger different, often stronger immune responses and cytokine profiles in mice compared to humans (Iwasaki and Medzhitov, 2004). This has been attributed to the differences in cellular expression of TLR9 in the two species. In mice, TLR9 is expressed in essentially all dendritic cell (DC) populations (conventional [cDC] and plasmacytoid [pDC]), macrophages, and all B cell subsets. In humans however, TLR9 expression is limited to pDC and some B cell subsets, and these are the main cells directly activated by CpG ODN (Hornung et al., 2002). All sheep B cells express TLR9 and are directly activated by CpG ODN (Booth et al., 2010). In pigs, pDCs are the main source of IFNα in blood mononuclear cells stimulated with CpG ODN (Guzylack-Piriou et al., 2004). Given the similar patterns of cellular expression of TLR9, it is not surprising that responses to CpG ODN are similar in humans and large animals, but less dramatic than in rodents. In this regard, it is thought that large animals can be valuable as animal models for studies exploring the application of CpG in humans. In addition, large animals present unique opportunities to explore the immunobiology of TLR9 which would otherwise be difficult to perform in humans for ethical reasons, and in mice due to the rather small body size. Most in vitro studies with CpG ODN have been done using cells isolated from blood of humans. While these studies have yielded valuable information, they only provide a narrow view given that blood contributes only a small proportion of immune cells in the body. Furthermore, the cellular composition of blood can vary considerably depending on the host physiologic status. Therefore, assessing how cells from other tissues respond to CpG stimulation would provide valuable insights. In large animals, large numbers of cells can be isolated from lymphoid tissues such as lymph nodes and intestinal Peyer's patches. In initial studies, we observed that cells isolated from peripheral lymph nodes responded strongly to CpG ODN (Booth et al., 2007), while cells from intestinal Peyer's patches responded poorly to stimulation with CpG ODN (Booth et al., 2009). As discussed in a separate article in this issue (Booth et al., 2011), these poor responses were not due to the inability of Peyer's patch cells to respond to CpG, but due to suppression by a regulatory B cell population present in this tissue (Booth et al., 2009). Therefore, the tissue environment can have a dramatic influence on cellular responses to CpG ODN and presumably to other TLR agonists.

3. Immunoprotection against infectious diseases

CpG activates innate immunity to protect against a variety of pathogens. Indeed numerous investigators have shown that in mouse models, CpG can protect against viral, bacterial and protozoal infections (reviewed in Krieg, 2006). In most of these models, pre-treatment with CpG ODN is the most effective in protection, while post-exposure therapy is generally ineffective against rapidly progressive acute infections (Krieg, 2006). The duration of protection varies from a few days to two weeks, but this could be extended to months by repeated treatments (Klinman et al., 1999). However, only a few studies have evaluated the ability of CpG ODN to stimulate innate immunity and protection against infections in domestic animals (summarized in Table 1).

Pretreament of newborn lambs with CpG ODN reduced virus shedding in bovine herpesvirus-1 (BHV-1) and bovine paraifluenza-3 virus (PI3) infections (Nichani et al., 2006, 2010). This protective effect was transient and only lasted for a few days, and coincided with increased levels of serum 2′5′A synthetase activity (Nichani et al., 2006, 2010). Also the effective dose of CpG ODN could be reduced by 80% when the ODN was formulated in an oil-in-water emulsion, which presumably provides a depot effect (Nichani et al., 2007).

Perhaps where CpG ODN has shown the most potent effects is in protecting chickens against bacterial infections (Table 1). Pretreatment of chickens with CpG ODN, followed by experimental challenge with *Escherichia coli* significantly improved survival (Gomis et al., 2003). Protection against *E. coli* septicaemia coincided with local recruitment of cells at the local site of injection (Gomis et al., 2003), suggesting a potential mechanism of protection. Similarly, CpG protected chickens against *Salmonella typhimurium* and *S. enteritidis*, as well as some viral and protozoal infections (Table 1, and reviewed in Dar et al., 2009). Also, in ovo administration of CpG ODN to embryos was effective in reducing mortality against subcutaneous