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Research paper

Cytokine production and proliferation upon in vitro oligodeoxyribonucleotide stimulation of equine peripheral blood mononuclear cells

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ABSTRACT

Synthetic oligodeoxyribonucleotides (ODN) may prove useful immune modulators in equine medicine. It is however important to assess the effects of each specific ODN in the species it is intended to be used in. The present study therefore aimed to evaluate some ODN for induction of cytokine production; i.e. type I interferons (IFN), IFN-γ, tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β), and proliferation of equine peripheral blood mononuclear cells (PBMC). A panel of four ODN containing unmethylated cytosine-guanosine sequences (CpG) was used: ODN 1 and ODN 8 representing A-class; ODN 2006 representing B-class and ODN 2395 representing C-class-ODN. In addition, two ODN where CpG-motifs were reversed to GpC were included; ODN 2137 otherwise identical to ODN 2006 and ODN 5328 otherwise identical to ODN 2395. Cytokine concentrations were measured in cell culture supernatants after 24 h of induction and proliferation was determined after 72 h of induction. Each ODN was tested with PBMC from at least 5 individual horses with and without the addition of lipofectin to cell cultures.

Type I IFN, IFN-γ and TNF-α production was readily induced by ODN 1, ODN 2006 and ODN 2395 both in the presence and absence of lipofectin and all three types of ODN induced similar levels of cytokines. Proliferation of PBMC was clearly induced by ODN 2006 and ODN 2395 while ODN 1 only induced low-level proliferation. The levels of proliferation induced were not influenced by the presence of lipofectin. TGF-β production was not induced by any of the tested ODN. ODN 8, ODN 2137 and ODN 5328 were largely inactive in all assays. Thus, responses seemed dependent on or increased by CpG-motifs but presence of CpG-motifs did not necessarily confer activity since ODN 8 was inactive despite its CpG-motifs.

Taken together, with equine PBMC distinctions in induction of different leukocyte functions between A-, B-, and C-class ODN were less obvious than what has been observed for human cells. These observations further stress the presence of species differences in ODN-induced responses.

Abbreviations: CI, confidence intervals; CpG, unmethylated cytosine–guanosine sequence; G, guanosine; MDBK, Madin–Darby bovine kidney; ODN, oligodeoxyribonucleotides; pDC, plasmacytoid dendritic cells; TLR, toll-like receptor; VSV, vesicular stomatitis virus.

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1. Introduction

Taking advantage of the innate immune recognition of DNA using short synthetic oligodeoxyribonucleotides (ODN) in order to evoke immune activation is nowadays a well-known concept. Indeed, ODN are being tested for use as e.g. vaccine adjuvants and as infection, cancer and allergy therapeutics (Agrawal and Kandimalla, 2007; Klinman, 2004; Krieg, 2006). Hence, there are several areas in equine medicine that could benefit from ODN induced immune stimulation. The optimal ODN for an application is dependent on a number of factors important for the responses elicited upon ODN stimulation.

The presence of an unmethylated cytosine followed by a guanosine (CpG) in the nucleotide sequence and the flanking bases of the CpG, i.e. the usually hexameric "CpG-motif" were early on pointed out as prime mediator of immune activating effects of DNA, and ligand of the DNA receptor toll-like receptor 9 (TLR9; Krieg, 2002, 2006). However, numerous reports of non-CpG mediated effects of both phosphodiester and phosphorothioate ODN exist (Bartz et al., 2004; Bernard and Phipps, 2007; Elias et al., 2003; Haas et al., 2008; Magnusson et al., 2001; Roberts et al., 2005; Vollmer et al., 2004b). Moreover, non-CpG mediated immune cell activation by ODN has been observed as TLR9-dependent as well as TLR9-independent (Bernard and Phipps, 2007; Chiu et al., 2009; Roberts et al., 2005; Takaoka et al., 2007; Unterholzner et al., 2010; Vollmer et al., 2004b). It has also been proposed that CpG-dependency is restricted to ODN with phosphorothioate backbones and not valid for ODN with phosphodiester backbones and that for the latter ODN the 2′deoxyribose sugar of the DNA backbone is responsible for TLR9 activation (Haas et al., 2008). Nonetheless, CpG-dependency has been observed for some phosphodiester ODN e.g. upon stimulation of human (Magnusson et al., 2001), porcine (Domeika et al., 2004) and equine cells (Wattrang et al., 2005). Thus, the role of CpG in TLR9 activation is still not completely elucidated but in the majority of cases responses to ODN are increased by, if not dependent on, the presence of CpG-motifs.

Nucleotide backbone is likewise of importance for ODN stimulation, where phosphodiester is the naturally occurring form and is sensitive to degradation while phosphorothioate is nuclease resistant and so far most often used for clinical applications (Agrawal and Kandimalla, 2007). Moreover, the combination of the complete ODN nucleotide sequence, backbone chemistry and its capacity to form intra- and/or inter molecular structure may determine its immune activity. Using mainly human cells, three different classes of ODN have been identified with respect to general delineation and responses elicited (e.g. reviewed in Bauer et al., 2008; Blasius and Beutler, 2010; Ishii and Akira, 2006; Klinman, 2004; Krieg, 2006). A-class ODN (also called type D) consist of a central phosphodiester palindrome sequence containing one or more CpG-motifs with 5′ and 3′ poly-guanosine (G) sequences (≥4 G) with phosphorothioate backbone. The palindrome and poly-G sequences makes these ODN prone to higher order structures, e.g. via G-tetrad and duplex formation. A-class ODN are considered strong activators of plasmacytoid dendritic cells (pDC), resulting e.g. in high production of type I interferons (IFNs), while they are poor activators of B-cells. B-class ODN (also called type K) consist of a complete phosphorothioate backbone of a linear single stranded conformation usually with multiple CpG-motifs. These ODN are mainly considered strong activators of B-cells and driving the maturation of DC but have also been reported to activate NK-cells. C-class ODN have a complete phosphorothioate backbone with 5′ CpG-motif(s) and 3′ palindrome sequences. The palindrome sequences make these ODN prone to inter- and intra-molecular duplex formation. The C-class ODN are able to both induce high type I IFN production in pDC and activate B-cells. Explanations of the differences in activities between ODN classes have been put forward, e.g. it has been proposed that the ability to form secondary structures is crucial for type I IFN induction by A-and C-class ODN (Guiducci et al., 2006; Kerkmann et al., 2005; Wikström et al., 2007). It has further been shown that these physical forms of A- and C-class ODN confer localization to, and/or retention in, early endosomes of pDC that in turn has been hypothesized as the key to high type I IFN production (Guiducci et al., 2006; Honda et al., 2005).

In addition to general effects of ODN sequences and chemistry, species differences in ODN induced responses are also apparent (Bauer et al., 2001; Rankin et al., 2001). Thus, choosing ODN for clinical applications not only involves consideration of optimal activating properties but also demands evaluation in the intended animal species. In the horse, there are still only a limited number of reports on ODN induced immune activation. It has been shown that a number of mainly B-class ODN induced proliferation of equine PBMC (Rankin et al., 2001). B-class ODN 2135 induced increased IFN-α and IL-12p40 mRNA expression in monocyte-derived DC from adult horses (Flaminio et al., 2007). In foal PBMC, B-class ODN 2135 and ODN 2142 and C-class ODN 2395 induced increased IFN-γ, IL-6 and IL-12p35/p40 mRNA expression (Liu et al., 2009b) and in foal neutrophils these two B-class ODN, but not the C-class ODN, induced IL-8, IFN-γ, IL-6 and IL-12p35/p40 mRNA expression (Liu et al., 2009a). In cultures of equine bronchoalveolar lavage cells A-class ODN 2216 was considered the most active inducer of IFN-γ, IL-4 and IL-10 compared to several B- and C-class ODN (Klier et al., 2011). Horse keratinocytes were however not activated by a C-class ODN (Leise et al., 2010). Addition of B-class ODN 2007 to a commercial killed vaccine against equine influenza virus enhanced antibody responses in vaccinated horses compared to those vaccinated with the original vaccine (Lopez et al., 2006). We have earlier evaluated a panel of ODN including three different A-class ODN for induction of type I IFN and IL-6 in PBMC (Wattrang et al., 2005). As expected these A-class ODN induced production of both cytokines but high levels of cytokine activity required treatment of ODN with the cationic lipid transfection agent lipofectin which is contrary to findings in other species, e.g. pigs, where lipofectin treatment did no affect the levels of IFN-α induced by these ODN (Domeika et al., 2004; Wikström et al., 2007). Hence, the present study was undertaken in order to broaden the knowledge on ODN activity on horse cells by evaluating cytokine production, i.e. type I IFN, IFN-γ, TNF-α and TGF-β, and proliferation by cultured equine PBMC. Four different stimulatory ODN were used;