Stimulation of Cytokine Expression by Peripheral Blood Mononuclear Cells of Neonatal Foals Using CpGs

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Young foals are particularly susceptible to infectious diseases. Cytosine-phosphate-guanosine dinucleotide oligodeoxynucleotides can stimulate cytokine expression by peripheral blood mononuclear cells of young foals. This stimulation of innate immunity might aid in protecting foals against infectious diseases. Authors’ addresses: Equine Infectious Disease Laboratory, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4475 (Liu, Cohen, Nerren, Murrell, Martens); and Merial SAS Discovery, Lyon, France (Juillard); e-mail: ncohen@cvm.tamu.edu (Cohen). © 2008 AAEP. *Presenting author.

1. Introduction

Neonatal foals are susceptible to a variety of microbial infections that are generally associated with underlying immunodeficiencies in people and other animals, such as Candida albicans, Cryptosporidium parvum, Pneumocystis carinii, and Rhodococcus equi. These infections are rarer in adult horses than foals, suggesting an age-related deficiency in immunity. Innate and adaptive immune responses are diminished in human neonates, and these deficiencies predispose to infectious disease. Innate immune responses provide the first line of defense against infections and serve to instruct the development of adaptive immune responses. There is evidence that interferon γ (IFNγ), an important mediator of innate immune responses, is relatively deficient in neonatal foals, and it has been proposed that this deficiency biases foals away from a T helper 1 (Th1)-type response, thus rendering foals more susceptible to infection. Therefore, enhancing expression of IFNγ and other pro-inflammatory cytokines might help protect foals against infection. Cytosine-phosphate-guanosine (CpG) dinucleotide motifs found frequently in bacterial DNA induce a strong Th1-type immune response, and synthetic CpG oligodeoxynucleotides (CpG-ODNs) are used as adjuvants against bacterial infectious diseases, neoplasms, and allergic disorders. The objective of this study was to investigate the extent to which peripheral blood mononuclear cells (PBMCs) from foals could be stimulated by selected proprietary CpG-ODNs to express mRNA of the pro-inflammatory cytokines IFNγ, tumor necrosis factor α (TNFα), and interleukin (IL)-6, IL-8, IL-12p35, and IL-12p40 and to determine the extent to which fold changes in expression of mRNA for these cytokines differed between mares and foals.

2. Materials and Methods

PBMCs were collected from fresh, heparinized, venous blood from five healthy Quarter Horse foals at
14 and 56 days of age and from their dams when the foals were ~21 days old. The PBMCs were divided into six aliquots such that one aliquot of $1 \times 10^6$ was incubated with each of the four CpG-ODNs (50 μg/ml) for 24 h and one aliquot of $1 \times 10^6$ was incubated for 23 h in media only, followed by 1 h of incubation with a virulent isolate (i.e., an isolate bearing the 80- to 90-kb virulence-associated plasmid) of R. equi (strain ATCC 33701; $1 \times 10^7$ cells/well) as a positive control for expression of IFNγ, TNFα, and IL-6. The remaining aliquot of PBMCs was incubated with only media for 24 h as a negative control. After incubation, total RNA was extracted from the cells, reverse transcribed, and subjected to real-time polymerase chain reaction (PCR) to determine levels of expression of IFNγ, TNFα, IL-6, IL-8, IL-12p35, and IL-12p40.

Expression of each cytokine relative to baseline (i.e., cells incubated in media only for 24 h) was calculated for each CpG-ODN using the ΔΔCt method. To account for the repeated measurements on individual foals, mixed-effects models were used for analysis of data, with foal modeled as a random effect, and age, sex, and the reference for stimulatory CpG-ODNs was CpG 2041 (referred to as the control CpG). It was also possible to make inferences about differences relative to the unstimulated control by evaluating the intercept term for each model. A similar modeling approach was used for the mare data, except that age was not considered as a factor. For a given cytokine, an ANOVA was performed comparing the values of differences between mares and foals (using a model for the paired differences at 14 days and another model for the paired differences at 56 days) by CpG; post-hoc testing of differences among the various CpGs was performed using Tukey’s least significant difference method. A significance level of $p < 0.05$ was used for analysis.

3. Results

Relative to PBMCs that were unstimulated or stimulated with the control CpG-ODN (CpG 2041), each of the stimulatory CpG-ODNs induced significant ($p < 0.05$) mRNA of IFNγ, IL-6, and IL-12p40 from PBMCs from foals at 14 and 56 days and from mares. There was no significant induction of expression of TNFα, IL-8, or IL-12p35 by the CpG-ODNs relative to the control CpG-ODN in mares or foals. There were no differences in expression levels observed between foals that were 14 and 56 days of age, and there were no significant differences between mares and foals in fold changes in expression of any of the cytokines. The mRNA expressions of IFNγ, IL-6, and IL-12p40 stimulated by CpG-ODNs 2135 and 2142 were ~2-fold higher than those induced by CpG-ODN 2395, although the differences were significant only for IL-12p40 and IL-6.

4. Discussion

Because foals may become infected with a variety of pathogenic microbes early in life at a time when their immune system is relatively immature and before the time in which an effective adaptive immune response can be developed for protection, strategies to stimulate innate immune responses might provide effective protection for foals against infectious diseases. This protection might result both directly by enhancing innate responses and indirectly from the critical role that innate immunity plays in development of adaptive immune responses. Foals produce relatively less IFNγ, IL-6, and other cytokines shortly after birth.2,3,7 Results of this study indicate that CpG-ODNs can effectively stimulate mRNA expression of IFNγ, IL-6, and IL-12p40 by PBMCs of foals as young as 2 wk of age. Moreover, the magnitude of the relative fold change in expression of IFNγ by foals was at least as strong if not stronger than that observed in mares. These findings indicate that CpG-ODNs might be useful as immunomodulators or as potential adjuvants for vaccines to aid in preventing R. equi pneumonia and other bacterial diseases of neonatal foals.

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References