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# Basal and stimulus-induced cytokine expression is selectively impaired in peripheral blood mononuclear cells of newborn foals

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ABSTRACT

Neonates are thought to be generally deficient in production of Th-1-associated cytokines at birth, and thereby more susceptible to bacterial infections. Using neonatal foals as a model, this study examined the age-dependent maturation of both basal and stimulus-induced immune responses, as reflected by the expression of a panel of Th-1-associated and pro-inflammatory cytokines. Results showed that although the basal production of IFN-γ and IL-6 was impaired ($P < 0.05$) in PBMCs of neonatal foals at birth, the basal production of IL-8, IL-12(p35/p40) and IL-23(p19/p40) were either in excess of or comparable to that of older foals. In response to *Rhodococcus equi* and CpG-ODN stimulation *in vitro*, PBMCs of neonatal foals showed increased ($P < 0.05$) expression of IFN-γ and IL-6, and preferentially increased expression of either IL-23(p19/p40) with *R. equi* stimulation or IL-12(p35/p40) with CpG-ODN stimulation. The magnitude of these stimulus-induced responses (except for IL-23p19), were significantly ($P < 0.05$) less for newborn foals than for older foals. The selective impairment of age-dependent basal and stimulus-induced cytokine expression by newborn foals may reflect the different functional state of various TLR pathways in newborns, and be directly associated with their age-dependent susceptibility to infection. Our results indicate that CpG-ODNs can selectively stimulate deficient cytokines ($P < 0.05$) from PBMCs in newborn foals *in vitro*, suggesting immunoprophylactic or therapeutic potential of CpG-ODNs.



## 1. Introduction

Neonates are highly susceptible to bacterial infections [1]. Because of deficiencies in their adaptive immune responses, neonates rely to a significant extent on innate immunity for protection against infections [1,2]. Innate immune responses of neonates have been assumed to be impaired or diminished due to their reduced expression of Th-1-cell-associated cytokines such as IFN-α, IFN-γ and IL-12 [2]. The discovery of several general families of innate immune receptors during the past decade has provided a new understanding of innate immunity. Innate immune receptors, including Toll-like receptor (TLRs) and non-TLR receptors, mediate the host recognition of pathogens [3,4]. The TLRs are important initiators of innate immunity, recognizing conserved pathogen-associated molecule patterns (PAMPs). Stimulation of immune cells with PAMPs causes recruitment of adaptor proteins with resultant triggering of downstream signaling cascades, followed by the production of pro-inflammatory cytokines and chemokines [5]. The release of these molecules, such as IFN-γ and IL-6, serves as a biomarker of the cellular response to the activation of the innate immune system.

Based on their primary sequences, TLRs can be divided into several subfamilies, each of which recognizes specific PAMPs. Progress in the past decade has revealed 10 TLRs (TLR1-TLR10) in humans, 3 of which play roles frequently reported in recognizing major bacterial cell components. TLR2 participates in the recognition of Gram-positive bacteria by sensing a variety of microbial components, including lipoprotein and peptidoglycan. Examples of bacteria that stimulate the TLR2 pathway include the group B *Streptococcus*, *Listeria monocytogenes*, *Mycobacterium tuberculosis* and *Rhodococcus equi* [5–8]. TLR4 mediates the recognition of lipopolysaccharide (LPS), the primary component of the outer membrane of Gram-negative bacteria [5–7]. TLR9, on the other hand, recognizes bacterial DNA, which is rich in unmethylated CpG motifs [9–12]. Synthetic CpG oligodinucleotides (CpG-ODNs) can mimic the immunostimulatory effects of native bacterial DNA, and activate multiple types of immune cells including peripheral blood mononuclear cells (PBMCs), B lymphocytes, macrophages, dendritic cells, and NK cells [12–16].

In spite of recent progress in our understanding of TLRs, the functional state of various TLR pathways in newborns, especially at birth, is still largely unknown [6]. Several studies have suggested

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that basal TLR expression in full-term neonatal blood monocytes is similar to that of adults [6,7]. The TLR-mediated production of cytokines by neonatal monocytes, however, is very different in newborns compared to that of adults [7,17]. This reduced gene expression and the impaired anti-microbial response in neonates, presumably resulting from a defect in TLR-mediated intracellular signaling, has been proposed to be to be the major deficiency in the innate cellular immune response of newborns. Other studies, how-

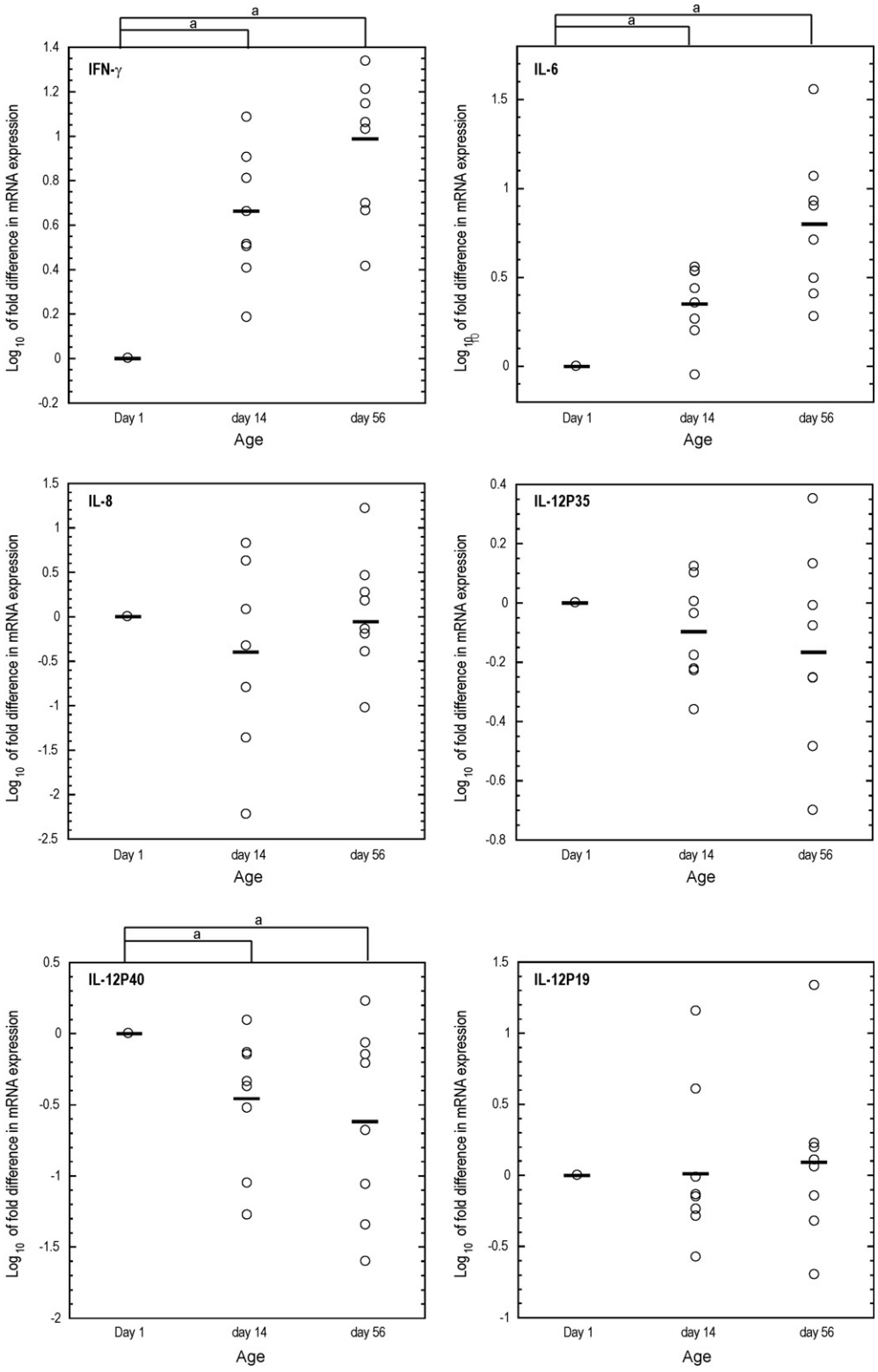


**Fig. 1.** Basal mRNA expression of IFN-γ, IL-6, IL-8, IL-12p35, IL-12p40 and IL-12p19 in PBMCs collected from foals ($n = 8$) on day 1, day 14 and day 56 of life. Note that the day 1 value for each foal was the referent category and assigned a value = 1 (see Section 2.6 for details). Total mRNA was isolated after incubation of the cells for 24 h in MEMα media, and relative expression was quantified by real-time RT-PCR. Each circle represents the result from an individual foal. Mean value from each group of individual foals is represented as a horizontal line. Relative to the day 1 group, age groups that show significant differences ($P < 0.05$) are marked on top of the figure with an "a".