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Retinoic acid-mediated anti-inflammatory responses in equine immune cells stimulated by LPS and allogeneic mesenchymal stem cells

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ABSTRACT

Retinoic acid (RA), an active metabolite of vitamin A, has shown potential therapeutic immunomodulatory properties. Allogeneic mesenchymal stem cells (MSCs)-based therapy is an effective approach to induce tissue healing and regeneration in many equine orthopedic conditions. However, MSCs-based therapies induced inflammatory responses *in vivo*. This study aimed to: 1. Determine the effect of RA cell culture treatment on inflammatory responses of lipopolysaccharides (LPS)- and allogeneic MSCs-stimulated peripheral blood mononuclear cells (PBMCs). 2. Determine the effect of RA on stimulated MSCs viability and morphology. Allogeneic MSCs-stimulated PBMCs had significant decreases in the anti-inflammatory cytokines (IL-10, IL-1ra, TGF-β1), increases in the pro-inflammatory mediators (IL-1β, IL-6, TNF-α, SAA), and increases of CD14 and MHC II percent positive cells compared to LPS- and non-stimulated PBMCs. Retinoic acid treatment of LPS- and allogeneic MSCs-stimulated PBMCs counterbalanced the induced inflammatory responses. Moreover, RA significantly improved the viability and morphology of stimulated MSCs. These findings highlighted the potential complications of equine allogeneic MSCs-based therapies and the immuno-modulatory effect of RA on equine stimulated cells. In conclusion, the use of RA to ameliorate allogeneic MSCs therapy associated inflammation may offer advantages that would require further investigations.

1. Introduction

Retinoic acid (RA), a vitamin A metabolite, is known for regulating a wide range of immune responses (Manicassamy and Pulendran, 2009; Napoli, 1999). This owed to its role in immune cells' proliferation, differentiation and functions (Noa, 2000). Multiple studies elucidated the effect of RA on different immune cells including macrophages (Kim et al., 2004), dendritic cells (DCs) (Geissmann et al., 2003), B cells (Di Caro et al., 2013) and T cells (Mucida et al., 2007). Exogenous RA treatment has also been reported to skew the immune status toward a tolerogenic status (Di Caro et al., 2013; Erkelens and Mebius, 2017). Moreover, RA is known for its immune suppressive and anti-inflammatory properties that nominated it as a therapeutic for inflammatory bowel diseases (Hong et al., 2014; Kang et al., 2009) and neuroinflammatory disorders (Van Neerven et al., 2010). Other research reports however, have proposed a contrary role for RA in the immune response. For instance, RA has shown to execrate T cell pro-inflammatory responses (Gatica et al., 2012; Paquette et al., 1996) and to worsen tissue inflammation (Saurer et al., 2007). Whether RA can evoke tolerogenic or pro-inflammatory effects remains an active area of investigation.

Toll-like receptors (TLRs) agonists' stimulation is the selected inflammatory model to mimic many clinical disease situations in horses. Equine bone marrow and peripheral blood mononuclear cells showed severe incomparable responses to different TLRs ligands' stimulation *in vitro* and *in vivo* (Hussein et al., 2016a; Leatherwood et al., 2016; Nieto et al., 2009). An important role of RA in TLRs signaling has been previously described and reviewed (Erkelens and Mebius, 2017; Hall et al., 2011). The TLRs mediated signaling was shown to induce Aldehyde dehydrogenase 1a (ALDH1a) expression in DCs and macrophages, the enzyme of which expression is essential for production of RA by these cells to modulate T and B cells inflammatory responses (Manicassamy et al., 2009; Uematsu et al., 2008). Toll like receptors' signaling has also been implicated in human mesenchymal stem cells (MSCs) migration and immune-modulating responses *in vitro* (Tomchuck et al., 2008).

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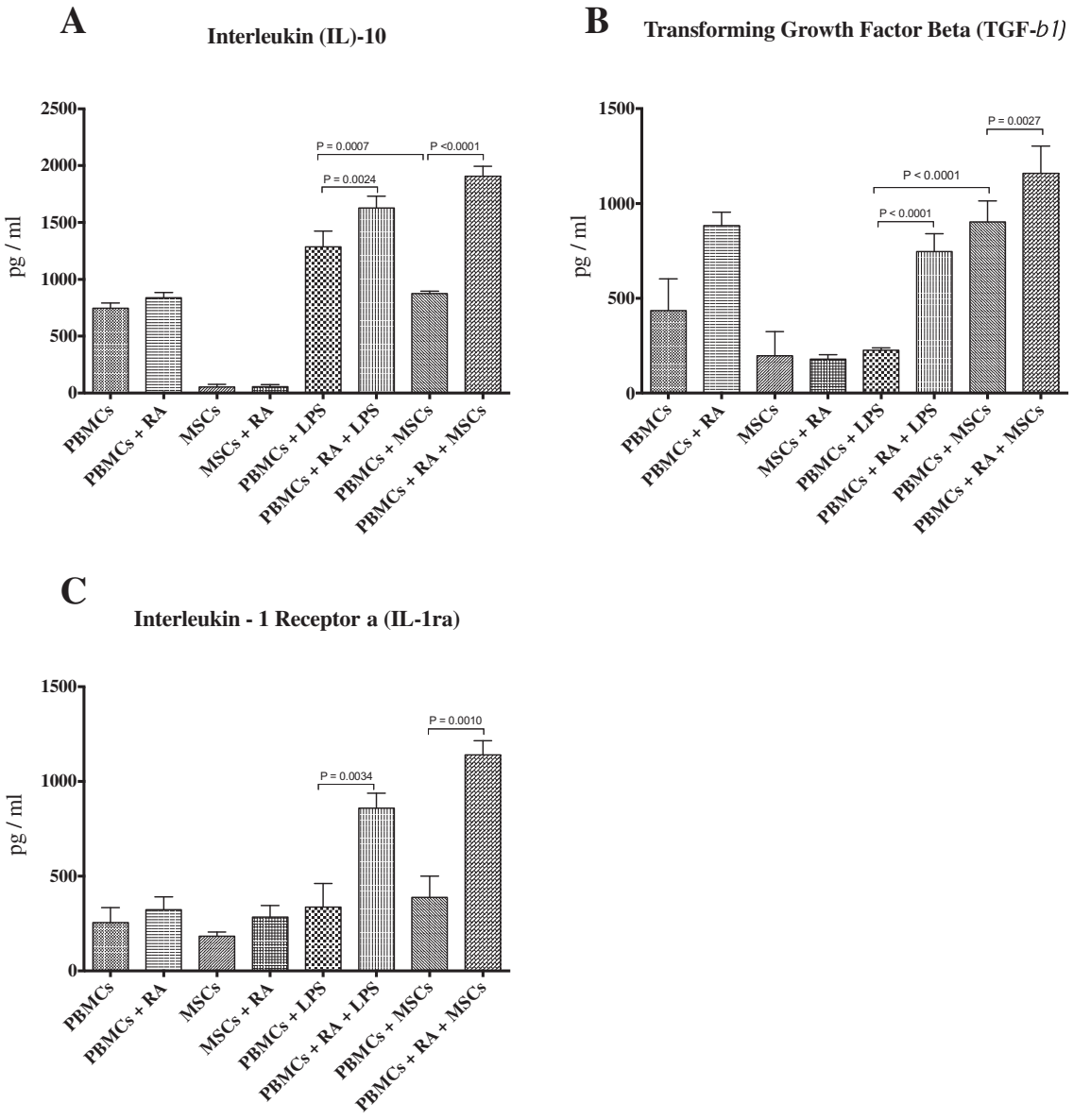

Fig. 1. Retinoic acid (RA) increased the anti-inflammatory mediators release by lipopolysaccharides (LPS)- and allogeneic mesenchymal stem cells (MSCs)-stimulated peripheral blood mononuclear cells (PBMCs). Different experimental conditions were performed using combinations of PBMCs, MSCs, RA, and LPS, and maintained in DMEM supplemented with 10% FBS for 48 h at 37 °C and 5% CO_2. The culture supernatants were then collected for analysis. The protein expression levels of interleukin (IL)-10 (A), Transforming growth factor (TGF)-β1 (B), and IL-1ra (C) were quantified using ELISA. Data represent mean ± standard error of the mean (SEM). Statistical comparison results are denoted in the graphs.

Allogeneic MSCs therapy has constituted a revolutionary therapeutic in horses. The application of this relatively novel therapeutic strategy for treatment of equine orthopedics, ischemic, inflammatory, and neurologic disorders has been reviewed in many research reports, and increasingly moving to commercial clinical use (Koch et al., 2009; Schnabel et al., 2013; Taylor et al., 2007). Although allogeneic MSCs have shown to be immune tolerated *in vitro* (Klyushnenkova et al., 2005; Le Blanc et al., 2003), these cells have shown to induce initial inflammatory/immune response upon transplantation in mice (Nauta et al., 2006), pigs (Poncelet et al., 2007), and equine (Pigott et al., 2013b). Interestingly, RA has also shown to promote MSCs differentiation to different mesenchymal lineages (Portmann-Lanz et al., 2006; Ventura et al., 2007; Zhang et al., 2010).

Considering the biological effect of RA and the increasing evidence that it may attenuate inflammation, we hypothesized that RA may have beneficial effects through modulating different immune responses in lipopolysaccharides (LPS)- and allogeneic MSCs-stimulated PBMCs. This study aimed to determine the effect of RA cell culture treatment of LPS- and allogeneic MSCs-stimulated peripheral blood mononuclear cells (PBMCs) on the anti-inflammatory mediators (Interleukin [IL]-10, IL-1ra, transforming growth factor [TGF]-β1), the pro-inflammatory mediators (IL-1β, IL-6, tumor necrosis factor (TNF)-α, serum amyloid A [SAA], CD14, MHC molecules) responses, and to investigate the effect of RA on stimulated MSCs viability and morphology.

2. Materials and methods

All experimental procedures were approved by the Veterinary Research Division of the National Research Centre, Egypt. Experimental animals' use and care were carried out according to the guidelines of the animal care statements of the Ethical Committee of the National Research Centre and Alexandria University.

2.1. Bone marrow aspiration and MSCs isolation

Sternal bone marrow was aspirated from 3 apparently healthy