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Research paper

Effect of hay dust extract and cyathostomin antigen stimulation on cytokine expression by PBMC in horses with recurrent airway obstruction

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ABSTRACT

Equine recurrent airway obstruction (RAO) is an inflammatory, obstructive airway disease induced by exposure of susceptible horses to inhaled organic dust particles. The immunological process underlying RAO is still unclear. Previous studies have shown that RAO is linked to the Interleukin-4 receptor (IL-4R) gene in one Warmblood family (F1), but not in another (F2). It has also been shown that in F1, but not in F2, RAO is associated with resistance against parasites, suggesting that this association may have an immuno-genetic basis. Therefore, we hypothesized that the T helper (h)1/Th2/regulatory (Treg) cytokine profiles of RAO-associated antigen- and parasite-antigen-stimulated peripheral blood mononuclear cells (PBMC) differ between RAO-affected and healthy horses depending on their genetic background. In our study, PBMC from 17 RAO-affected and 14 healthy control horses of F1 and F2 were stimulated for 24 h with antigens relevant to RAO [hay dust extract (HDE), *Aspergillus fumigatus* extract (AFE) and lipopolysaccharids (LPS)]; cyathostomin extract (CE) and recombinant cyathostomin antigen (RCA) or with concanavalin A (ConA). Total mRNA levels of IL-4, IL-4R, IL-13, interferon (INF)-γ and IL-10 were examined by qRT-PCR. Stimulation with either HDE or RCA resulted in significant differences in IL-4R mRNA levels between RAO-affected and control horses in F1, but not in F2. For IL-10 mRNA expression, a significant difference between RAO-affected and control horses in F1 but not in F2 was observed only following stimulation with HDE. In contrast to HDE, stimulation with CE resulted in a significant difference of IL-10 mRNA expression level between RAO-affected horses of F2 and healthy horses of F1. No significant differences were detected upon stimulation with any of the other challenge agents. These findings indicate that the immunological response, specifically IL-4R expression, in response to hay dust and cyathostomin antigens, differs between RAO-affected and healthy horses depending on their genetic background. This study shows that analysis of PBMC reveals systemic changes associated with RAO and helps to elucidate immunological pathways involved in this disease.

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1. Introduction

Equine recurrent airway obstruction (RAO), or heaves, is an inflammatory airway disease characterized by periods of airway obstruction caused by hypersensitivity to inhaled organic dust particles (Robinson et al., 1996; Leclere et al., 2011). Seven to twenty percent of horses in the Northern hemisphere and other temperate climates suffer from RAO (Baleri, 2008; Leclere et al., 2011). Affected horses are typically middle-aged and older (McPherson et al., 1979; Leguillette, 2003; Swinburne et al., 2009). RAO-affected horses develop bronchospasm, airway hyperreactivity, mucus accumulation and neutrophilia leading to airway obstruction (Robinson et al., 1996; Gerber et al., 2004). Typical clinical signs of RAO include chronic cough, respiratory distress, nasal discharge and abnormal lung sounds (Robinson, 2001).

The specific antigens involved in triggering RAO have not been clearly identified so far. However, several studies have shown exacerbation of clinical signs in RAO-affected horses stabled with straw bedding and fed hay (Woods et al., 1993; Robinson et al., 1996; Vandenput et al., 1997; McGorum et al., 1998) or following aerosolization of hay dust suspensions and mold spores (McPherson et al., 1979; Derksen et al., 1988; Mc Gorum et al., 1993; Pirie et al., 2003a, 2003b; Leclere et al., 2011). Hay is the most common source of organic dust in stables, and contains inhalable moulds, mites, endotoxin and inorganic particles. Non-specific responses to endotoxin also play a role in RAO (Pirie et al., 2003a, 2003b).

Although immune parameters such as cytokine expression levels have been determined in cells from bronchoalveolar lavage fluid (BALF) or peripheral blood, the specific immunological basis for RAO is still controversially discussed. In agreement with the hypothesis that a local allergy-like response is involved in RAO, a number of studies found an increased frequency of Interleukin (IL)-4 and IL-13-expressing T helper (h)2 cells or of the Th2-driving cytokine TSLP in BALF cells (Cordeau et al., 2004; Lavoie et al., 2001; Lavoie-Lamoureux et al., 2010; Klukowska-Rötzler et al., 2012a). Other studies reported a predominant Th1 response, characterized by interferon (INF)-γ (Ainsworth et al., 2003; Debrue et al., 2005), or revealed a combined Th1 and Th2 immune response (Beadle et al., 2002; Horohov et al., 2005). However, expression of cytokines following stimulation of peripheral blood mononuclear cells (PBMC) with potential allergens has not been investigated so far in RAO-affected horses.

RAO shows a familial predisposition and a complex mode of inheritance (Marti et al., 1991; Ramseyer et al., 2007; Gerber et al., 2009). We have recently identified two Swiss Warmblood families (family 1 and family 2) with a high prevalence of RAO (Ramseyer et al., 2007). In family 1 (F1), RAO follows a recessive mode of inheritance and an association with equine chromosome 13 (ECA13), on which the IL-4R gene is located (Jost et al., 2007; Gerber et al., 2009; Klukowska-Rötzler et al., 2012b; Racine et al., 2011). In contrast, RAO in family 2 (F2) shows an autosomal dominant mode and is linked to ECA15 (Gerber et al., 2009; Swinburne et al., 2009). Despite the genetic heterogeneity between family 1 and family 2, the clinical RAO phenotype did not differ between the two families (Laumen et al., 2010). Interestingly, recent studies have found an association between RAO and resistance to parasitic infections in family 1, but not in family 2 (Neuhaus et al., 2010; Bründler et al., 2011). An important aspect of this relation is that Th2 genes, including the IL-4R, are associated with human asthma (Ober et al., 2000; Tachdjian et al., 2009) as well as with defense against parasitic nematodes in humans and animals (Scales et al., 2007). Parasitic nematodes of the group Cyathostominae ubiquitarily infect almost all grazing horses. This group consists of approximately 50 species, all of which have similar life cycles that involve encystment of larval stages in the large intestinal wall (McWilliam et al., 2010). Horses with large encysted larval burdens often have low or negative fecal egg counts (Paul, 1998). Recently, two antigen complexes derived from cyathostomin encysted larvae were identified (Dowdall et al., 2004). A recombinant protein of one of these two antigens was revealed as a reliable diagnostic marker for the estimation of cyathostomin encysted larval burdens (McWilliam et al., 2010).

Conclusively, we hypothesized that genes encoding for specific immunological pathways, in particular those involved in Th1, Th2 and Treg type pathways, are differentially expressed between RAO-affected and healthy horses depending on their genetic background relevance to susceptibility to RAO and resistance against parasites. The aim of the present study was to determine the in vitro cytokine (IL-4R, IL-4, IL-13, INF-γ and IL-10) gene expression levels in PBMC after culture with stimuli relevant to RAO and cyathostomin infections in RAO- affected horses and healthy controls in two families characterized with respect to their genetic background.

2. Materials and methods

2.1. Horses

Half-siblings of two RAO-affected Swiss Warmblood stallions were included in the study. Horse Owner Assessed Respiratory Signs Index (HOARSI) scoring system based on a standardized questionnaire, was used to grade RAO severity as described previously by Ramseyer et al. (2007). The horses were scored according to HOARSI 1–4 from healthy to severely affected, with HOARSI 3 and 4 corresponding to the presence of the RAO phenotype, as shown previously (Laumen et al., 2010). The case definition for HOARSI 3 is abnormal breathing, regular or frequent coughing, or both. HOARSI 4 is defined by poor performance in addition to the same clinical signs as HOARSI 3. The classification refers to the period when the horses were exhibiting their most severe clinical signs. At the time of the study, the RAO horses were kept in a "low dust" environment and were in complete or 'partial' remission showing either no or only mild clinical signs (mainly mildly increased breathing effort).

A total of 31 adult horses (21 geldings, 10 mares) were divided into four subgroups: family 1, RAO-affected animals (F1-A, $n=9$) and healthy controls (F1-C, $n=6$) and family 2, RAO-affected animals (F2-A, $n=8$) and healthy controls (F2-C, $n=8$). All horses were $\geq$13 years of age,