Introduction: Degenerate joint disease is prevalent in race horses. This results in a significant economical impact on the equine industry. Recently, a novel treatment for degenerate joint disease has been developed that involves the use of autologous conditioned serum (ACS) [1]. This treatment involves incubating the patient's own serum with glass beads overnight and re-injecting the treated serum into the affected joints. It is believed that this treatment upregulates beneficial cytokines, especially interleukin-1 receptor antagonist (IL-1ra) which is the natural inhibitor of IL-1 [2]. IL-1 has been shown to be elevated in degenerate joint disease and can stimulate cartilage breakdown [3]. In this study, we analyzed the serum of race horses that were treated with ACS to determine what cytokines were induced with treatment.

Materials and Methods: Horse serum: Race horses (n=6) that were diagnosed with degenerate joint disease and had clinical lameness were selected for treatment. Serum was collected and treated with Orthogen as described by manufacturer. An aliquot of pre-treatment and treated serum were kept and frozen at -80°C for biochemical analysis.

Cytokine profiling: Human and mouse cytokine (R&D) array blots were used to profile 36 different cytokines simultaneously. Horse serum (500 μl) was incubated with the cytokine arrays as described by the manufacturer.

IL-1ra ELISA: A commercial mouse IL-1ra ELISA (R&D) was used to assay horse serum for IL-1ra levels.

Results: We measured the levels of 36 different cytokines in pre-processed and treated ACS from race horses which had clinical lameness (Fig. 1). We did not detect increases in IL-1ra levels in all of the samples analyzed. We did however detect slight increases in IL-8, IL-13, MIF and PAI-1 in treated serum. The treatment did not stimulate IL-1, IL-6 or TNF. To confirm the IL-1ra levels, we used a mouse IL-1ra ELISA that was reported to be more accurate by Frisbie et al. [3] and confirmed that IL-1ra were not stimulated by ACS treatment. All the horses treated with ACS had improvement in clinical lameness.

Discussion: ACS is a novel treatment that has been reported to stimulate the patients' own serum to upregulate beneficial cytokines such as IL-1ra. It has been reported to be used in over 30,000 humans and 3000 horses [4]. In this study, we measured the changes in horse serum that were incubated with glass beads. We did not detect an upregulation of IL-1ra in the processed serum. This is different from results that have been reported recently by Frisbie et al. [4]. They reported that IL-1ra levels were significantly greater in ACS treated serum. We did detect an increase in IL-13 levels in ACS serum. IL-13 has been shown to be chondroprotective [3,5] and can block collagen degradation [5]. Although we did not detect an increase in IL-1ra levels in ACS, the treated horses all improved in their clinical lameness, suggesting that there is a beneficial affect of ACS treatment. Further studies in the levels of IL-13 and other cytokines profiled may lead to understanding the mechanism of action of ACS treatment on horses.

References:

Fig. 1. Representative cytokine array blot of horse serum pre and post treatment. IL-1ra levels were always low and was not stimulated by treatment. Occasionally, other cytokines such as IL-13 were stimulated.