Introduction: Osteoarthritis (OA) is marked by an up-regulation of the inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF-α) (1). Competitive inhibition of the IL-1 and the TNF-α receptors has been a target for recombiant protein therapy. Recombinant forms of the IL-1 receptor antagonist protein (IL-1ra) and the soluble TNF-RI binding protein (sTNF-RI) have been approved for use to treat rheumatoid arthritis (Amgen Corp., Thousand Oaks, CA). Additionally, both the recombinant human IL-1ra and an autologous-derived IL-1ra have been evaluated for the treatment of OA, though to date, neither has received FDA approval (2). Inhibition of IL-1 and TNF-α with a single inhibitory cytokine has demonstrated limited clinically efficacy. A likely cause is the redundancy of the action of these two molecules (3). Therefore, an autologous protein serum that contains concentrated IL-1ra and sTNF-RI would be desirable for evaluation as a treatment for OA.

A previously published target dose for autologous IL-1ra used to treat OA was 6 ng per injection (4). No such dose has been described for autologous sTNF-RI. A novel method to collect IL-1ra from platelet-rich plasma (PRP) following a 24-hour incubation in a plasma concentration device with polyacrylamide beads was previously described and was shown to exceed this dose (5). Incubation of less than 24 hours would improve the clinical utility of this method. Therefore, the primary purpose of this study was to determine the minimum time required to produce >6ng IL-1ra while also producing detectable levels of sTNF-RI. A secondary objective was to evaluate the production of several other inflammatory cytokines.

Materials and Methods: A 24-hour time course was performed analyzing the production of IL-1ra and sTNF-RI during incubation of a PRP with polyacrylamide beads at room temperature and at 37°C. Units of purified human IL-1α (anticoagulated with ACD-A) were used to complete the time course (2 units from Lampire Biological Laboratories, PA). PRPs were prepared using fresh blood from 5 human volunteers. The serum output was collected, modified plasma concentration devices (Plasmax®, Biomet Biologics, LLC, Warsaw, IN) were used to process the PRP with the polyacrylamide beads without any extended incubation time. Following PRP production, 3 ml of PRP were loaded into each of 9 modified plasma concentration devices (Plasmax®, Biomet Biologics, Warsaw, IN) and were incubated for 0, 2, 4, 6, and 24 hours at 37°C and at room temperature. Following incubation, the Plasmax® units were centrifuged at 2000 rpm for 2 minutes. The serum output was collected, and the volumes were recorded.

For baseline comparison, the whole blood (base) and remaining PRP was activated with 50 μl of bovine thrombin and 1M CaCl₂ (1,000 units/ml). The thrombin-activated samples were incubated for 30 minutes at room temperature. Following activation, the whole blood and PRP was centrifuged for 5 minutes at 3000 rpm. The serum volume was collected and recorded. All samples were measured for IL-1ra and sTNF-RI using ELISAs (R&D Systems, Minneapolis, MN).

Following the initial 24-hour time course, a shorter, second time course was performed using fresh blood from 5 human volunteers. The previously described procedure was repeated with modified Plasmax® units incubated for 0, 2, and 4 hours at room temperature. ELISAs were used to measure the concentration of the anti-inflammatory cytokines IL-1ra, sTNF-RI, and the pro-inflammatory cytokines TNF-α, IL-1β, IL-1α, and IL-10. Data is presented as mean ± standard deviation. Statistical significance was determined using a Student’s t-test (α=0.05).

Results: Incubation of PRP with polyacrylamide beads demonstrated a statistically significant increase of IL-1α over both the baseline and PRP samples (p=0.003). However, neither time nor temperature significantly increased the IL-1α production (Fig. 1). sTNF-RI production was detectable at all time points measured, but no differences between time points or temperature were seen with the banked blood (Fig. 1). These data suggest that the >6ng of IL-1ra was achievable by simply processing the PRP with the polyacrylamide beads without any extended incubation time. A second time course with fresh blood was repeated to confirm this result (Table 1). With fresh human blood, IL-1ra and sTNF-RI levels were significantly higher in the samples processed with the polyacrylamide beads than in the whole blood (p<0.001 and p=0.02 respectively). As seen in the earlier evaluation with banked blood, neither cytokine increased with incubation time.

Finally, pro-inflammatory cytokines were measured during the second time course (Table 1). Very little data was acquired from the IL-1β, IL-1α, or the TNF-α assays, as most of the readings were below the detection limit of the assay. These data suggest that the method of processing PRP with polyacrylamide beads does not significantly increase the concentration of pro-inflammatory cytokines that could possibly negate the effects of the IL-1ra and sTNF-RI.

Discussion: IL-1α produced using the modified Plasmax® after 0 hours of incubation was approximately 37 ng, which is at least 6 times greater than the acceptance criteria of 6 ng. The time course data suggest a lack of dependence on incubation time or temperature for the stimulation of IL-1ra and sTNF-RI. This data also contradicts an original theory that the polyacrylamide beads prompt the monocytes to transform into macrophages and begin producing anti-inflammatory cytokines (5). It is now more likely that the beads cause the cells present in the PRP to lyse upon contact, immediately releasing the cytokines into the concentrated plasma.

No increase in inflammatory cytokines was detected in this production method. It has been reported that the IL-1ra needs to be at least 10 to 1000-fold in excess over the IL-1β to inhibit the IL-1 biologic activity (6). In this study, the IL-1α was 4000-times greater than the IL-1β. However, one limitation to the present study was that the human donors were all healthy individuals, with normal levels of inflammatory cytokines. Additional studies on OA patients would need to be completed to determine the ability of these patients to produce IL-1ra and sTNF-RI, and to compare these values to those of the pro-inflammatory cytokines also produced.

References:
(3) Abbas AK, et al., Cellular and Molecular Immunology, Saunders, (2003) 243-274.