Introduction: There is a renewed interest in metal-on-metal bearing surfaces in total joint arthroplasty. However, increased levels of serum metal ions in patients with well-functioning metal-on-metal implants have been reported. Exposure to metal ions have been implicated in inducing osteolysis, local immune dysfunction and carcinogenesis. Induction of these pathologic processes has also been attributed to metal induced apoptosis. However, it is unclear whether metal ions induce apoptotic cell death. The present study investigated the cytotoxic effects of several metal ions. These ions are derived from metals that constitute the major components used in the design of orthopaedic implants. We tested the hypothesis that metal ions induce apoptotic cell death. To test our hypothesis, we used a human prototype T lymphocyte line-Jurkat T cells. This cell line is well accepted model for studying T cell function and apoptotic cell death.

Materials and Methods: Human prototype T lymphocyte line-Jurkat T cells were incubated with metal ions: titanium (Ti[III]), cobalt (Co[II]), chromium (Cr[VI]) or control medium alone. Cells were incubated at concentrations ranging from 1 ng to 100 µg/ml for a maximum of 72 hrs. at 37°C, 5% CO2. Cell viability was determined by trypan blue vital dye uptake and proliferative capacity was evaluated by 3H-thymidine uptake. Apoptotic indices were assessed by analyzing (1) induction of caspase 3 by Reverse Transcription Polymerase Chain Reaction (RT-PCR), (2) nuclear DNA fragmentation using the Hoechst stain, (3) DNA fragmentation using the Ladder electrophoretic technique, and (4) transmission electron microscopy. Statistical analysis was performed using the paired t test with Intercooled Stata 6.0 software. Data were obtained from three separate experiments (+ sd).

Essential Results: Co[II] and Cr[VI] induced cell death in a dose dependent manner. Incubation with concentrations greater than 10µg/ml (42µM) of Co[II] and 1µg/ml (4µM) of Cr[VI], induced caspase 3 expression, an enzyme that catalyzes DNA degradation as shown by RT-PCR analysis (Figure 1). Induction of caspase 3 was followed by nuclear fragmentation and condensation of chromatin by 48 hrs. The frequency of cells showing fragmented chromatin, blebbing and apoptotic bodies identified by Hoechst stain was greatest (~60%) with Cr[VI] (p<0.05, Figure 2) at day 1. TEM verified chromatin condensation and nuclear fragmentation (Figure 3, a=untreated control, b,c= apoptotic cells). DNA fragmentation was further verified by DNA electrophoretic analysis shown in lanes 2-3 (Figure 3 top panel, lanes 4-7 bottom panel) following treatment with metal ions. Figure 3 shows top panel, M=DNA markers, Co[II] lane 1= 10µg/ml, 24 hrs, lane 2=10µg/ml, 48 hrs, 3=10µg/ml, 72 hrs, lane 4= 100µg/ml, 24 hrs and lane 5 untreated control. Figure 3 shows lower panel Cr[VI], lane 1=untreated control, lane 2=10µg/ml, 6hrs, lane 3=1µg/ml, 24 hrs, lane 4=10µg/ml, 24 hrs, lane 5 = 1µg/ml, 48 hrs, lane 6= 10µg/ml, 48 hrs, lane 7=1 µg/ml, 72 hrs and lane 8=10 µg/ml, 72 hrs.

Discussion and Conclusions: The present study demonstrated for the first time that Co[II] and Cr[VI] induce T cell death and that the mode of cytotoxicity involves the apoptotic pathway. Cell death via apoptosis appears to be dependent on the type of metal alloy, the valency of the ions and upon the concentration as well as time of exposure. These studies suggest that chronic exposure to critical concentrations of metal ions could result in T cell apoptosis which in turn could lead to potential pathologic tissue response. Our study prompts increased awareness of the potential deleterious effects of metal ions released from prosthesis.

Acknowledgments: Supported by the Good Samaritan Hospital the Orthopaedic Rheumatology Gift Fund

INDUCTION OF APOPTOSIS IN THE T-LYMPHOCYTE JURKAT CELL LINE BY PROSTHETIC METAL IONS

** Kabata, T; *Khanuja, HS; *Polotsky, AV; *Hungerford , DS; ** Tomita, K; +*Frondoza, CG+*The Johns Hopkins University, Department of Orthopaedic Surgery, The Good Samaritan Hospital, 5601 Loch Raven Blvd. Baltimore Maryland, 21239, USA,
**Kanazawa University, Department of Orthopaedic Surgery, 13-1 Takaramachi, Kanazawa, 920-8641, Japan

Figure 1

Figure 2

Figure 3

Figure 4