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Metal Ions Activate Vascular Endothelial Cells and Increase Lymphocyte Chemotaxis and Binding

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Abstract

Metal on metal articulations in hip arthroplasty offer advantages, including lower volumetric wear compared to conventional metal on polyethylene bearings, and increased resistance to dislocation. Reports described early failures, with histologic features similar to a Type IV immune response. Mechanisms by which metal wear products cause this reaction are not completely understood. We hypothesized a mechanism through direct activation of endothelial cells (ECs) by metal ions, resulting in both vasculitis and accumulation of lymphocytes without prior immune sensitization. Effects of metal ions were evaluated using human ECs in culture. Alterations in chemotactic proteins IL8 and MCP1 were assessed, as was upregulation of the adhesion molecule ICAM-1 and lymphocyte binding to ECs. Cobalt increased secretion of IL8 and MCP1 significantly, and upregulated the expression of ICAM-1 in ECs compared to stimulation by chromium and controls. Binding of lymphocytes to ECs and transEC migration were both significantly increased by cobalt but not chromium. These findings suggest that cobalt contributes more to the activation of ECs and lymphocyte binding than chromium without an allergic response. Some of the adverse tissue reactions to implants with components made of cobalt–chromium–molybdenum alloys may be due in part to activation of the endothelium by metal ions.

Keywords

metal on metal; lymphocytes; endothelium

Concerns regarding the generation of wear particles in total joint arthroplasty from metal on polyethylene bearings have stimulated interest in alternative bearing surfaces.¹ The use of metal-on-metal (MoM) articulations in hip arthroplasty offers several potential advantages, including lower volumetric wear compared to conventional metal on polyethylene due to lower wear rates, as well as increased stability and resistance to dislocation. Despite some of the clinical advantages such as increased stability, there is growing evidence that MoM articulations can produce demonstrably increased levels of circulating metal ions including Co²⁺ and chromium (Cr³⁺).^{2–6} The increased metal ion levels can create a biologic reaction, consisting of perivascular lymphocytic infiltrates, termed aseptic, lymphocyte dominated, vasculitis associated lesions (ALVAL).^{7,8} Tissues surrounding failed implants often have characteristics of a Type IV hypersensitivity reaction, including vasculitis and the

perivascular accumulation of lymphocytes.⁸⁻¹³ But not all patients with these failed implants have demonstrated an immune response when challenged by metal ions that compose these implants suggesting other mechanisms.¹⁴ The impact of the increased circulating metal ions including Co^{2+} and Cr^{3+} on ECs is not completely understood. Activated endothelium induced transendothelial migration of lymphocytes is a process involving chemokine and cytokine release, for example, IL-8 and MCP-1.¹⁵⁻¹⁷ The release of cytokines and chemokines suggest that other, nonallergic, mechanisms may exist by which metal ions more directly affect implant loosening. We therefore investigated the possibility that a potential mechanism is through the direct activation of ECs when exposed to metal ions, and that the interactions between ECs and lymphocytes result in the transendothelial migration and accumulation of lymphocytes without the need for sensitization by metal ions.

The goal of this in vitro study is to assess the effects of Co^{2+} and Cr^{3+} endothelial activation, including lymphocyte chemotaxis, the secretion of chemokines (MCP1 and IL8), upregulation of binding proteins (MCP1), and transendothelial migration. As such, we propose that the endothelium is a potential regulatory feature in the development of the response to metal ions, and that such reactions might play a role in any MoM articulation in athroplasty, regardless of implant design.

METHODS

Cell Culture

Human umbilical vein ECs (HUVEC) (Lonza, Walkersville, MD) were grown in Endothelial Growth Medium (EGM), (Lonza, Walkersville, MD), with 2% fetal bovine serum (FBS), and 1% antibiotics. HUVEC were plated at a density of 5,000 cells/cm² in cell culture dishes and grown to confluency. Lymphocytes (Jurkat) (American Type Culture Collection [ATCC]), (Manassas, VA) were grown in RPMI 1640 medium plus 10% FBS and 1% antibiotics.¹⁸ Cells were maintained at 37°C. and 5% CO₂. Although derived from an immortalized line of lymphocytes, the use of Jurkat cells is a well-established model for Thy+ lymphocytes.¹⁰

Preparation of Ions

Chloride salts of both Co^{2+} and Cr^{3+} (Sigma, St. Louis, MO) were used in all studies, with equimolar concentrations of NaCl used as negative controls. Metal salts were dissolved in Dulbecco's Phosphate Buffered Saline (DPBS) and added as dilutions to the cell culture wells. Cobalt chloride (1 mM), chromium chloride (1 mM), and sodium chloride (1 mM) were added in fresh media according to the literature.^{19,20}

Cellular Toxicity

Cellular toxicity was determined through the release of LDH into the conditioned media (CytoTox 96® NonRadioactive Cytotoxicity Assay, Promega, Madison, WI). Cells were plated at 5,000 cells/cm² in 24 well cell culture dishes and grown to confluency. Cobalt chloride (1 mM), chromium chloride (1 mM), and sodium chloride (1 mM) were added in fresh media to the confluent cell culture. Metal concentrations were used as described in the literatures.^{19,20} After 6, 12, 24, and 48 h LDH release was assessed. Released LDH in culture supernatants is measured with a 30 min coupled enzymatic assay, which results in conversion of a tetrazolium salt into a red formazan product. Absorbance was recorded using a BioTek microplate reader at 490 nm.

Secretion of Chemokines

Alterations in the secretion of the chemokines interleukin 8 (IL8) and monocyte chemoattractant protein 1 (MCP1) were determined by ELISA (R&D Systems, Minneapolis, MN). HUVEC were grown in cell culture until confluent and the media was changed to new media containing the final desired concentration of cobalt chloride, chromium chloride and the negative control sodium chloride. Following incubation for 48 h, conditioned media were collected and frozen at 70°C for further analyses. ELISAs were read on a BioTek microplate reader at 450 nm.

Fluorescence Adhesion Assay

The adhesion assay was based on a kit from Cell Biolabs, San Diego CA. Lymphocytic Jurkat cells (1×10^6) were harvested in serum free medium. A fluorescent label, Leuko-Tracker, was added to a final concentration of 1×10^{-6} . The lymphocytes were incubated for 1 h at 37°C in an incubator. The cells were centrifuged at 1,000 rpm and washed with serum free media. HUVEC were grown to near confluence in black 24 well tissue culture plates suitable for reading on a fluorescent reader. HUVEC were incubated in EGM medium with cobalt chloride (1 mM), chromium chloride (1 mM), or TNF α (2 ng/ml) as a positive control. After 2 h, 250,000 fluorescent labeled lymphocytes were added to each well. The lymphocytes were allowed to settle onto the ECs for 3 h and then nonadherent cells were gently washed off three times with DPBS. The number of adherent lymphocytes was quantified on a Fluorostar Omega Microplate Reader, BMG Labtech at 480/520 nm.

Adhesion by Light Microscopy

HUVEC and Jurkats were grown as outlined above in the Fluorescent Adhesion section. The protocol remained the same except that a fluorescent tracker was not added. The cells were imaged and photomicrographs were taken using a Nikon TS1000 microscope. For cell counts an average of three fields were counted and quantified using the Nikon Element software program.

Immunohistochemistry

To visualize ICAM-1 expression ECs were plated onto chamber slides at an initial density of 25,000 cells/chamber. ECs were stimulated with cobalt chloride (1 mM), chromium chloride (1 mM), or TNF α (2 ng/ml) as a positive control for 24 and 48 h. Cells were fixed with 1% paraformaldehyde (10 min) and solubilized in 0.5% Triton X100 (5 min). Primary antibodies were applied using a primary rabbit antiICAM-1 antibody (Santa Cruz Biotechnologies, Inc., Santa Cruz, CA), in a 1:50 dilution followed by Alexa Fluor 488 labeled goat anti-rabbit IgG antibody (Invitrogen, Carlsbad, CA, 1:200 dilution). Nuclei were labeled with ToPro3 (Invitrogen, 1:1,000 dilution). The slides were sealed with an aqueous mounting media and stored at -20°C for subsequent analysis by confocal microscopy. Fluorescence intensity was measured by determining pixel intensity of each cell.

Western Blots

Following 4, 12, 24, and 48 h incubation with 1 mM cobalt chloride, 1 mM chromium chloride, 1 mM NaCl, and 2 ng/ml TNF α , protein was isolated from endothelial cells by sonication in 20 mM MOPS lysis buffer, pH 7.0, containing 2 mM EGTA, 5 mM EDTA, 30 mM NaF, and 0.5% NP40, (all from Sigma), phosphatase inhibitors (Phosphatase Inhibitor Cocktail, Sigma) and protease inhibitors (Roche, Indianapolis, IN). Protein concentration was determined using a BCA Protein Assay (Pierce Biotechnology Inc., Rockford, IL). Proteins were separated using 4-20% SDS-PAGE gels (BioRad, Hercules, CA). The proteins were transferred to nitrocellulose membranes and probed with rabbit anti-ICAM-1 antibody

(Santa Cruz Biotechnologies, Inc.). Horseradish peroxidase conjugated goat anti-rabbit IgG was then applied to the membranes. The protein bands were imaged using Amersham ECL Plus (GE Health Care, Piscataway, NJ). Images of band density in the autoradiograms were captured using Photo-studio software (ArcSoft, Frenont CA) and a flatbed scanner (Canon, Model N12204, Lake Success, NY). Band densities were analyzed using ImageJ software (NIH, Bethesda, MD).

Vascular Permeability Assay

Vascular permeability was evaluated using sections of rabbit carotid artery that were dissected free of associated soft tissues, cannulated using a 24 gauge catheter and flushed. The vessel was then harvested, flushed to remove all remaining cells, and tied off on one side. Solutions consisting of saline alone (1 ml), saline with blue marker dye (Patent blue, Sigma) (1 ml), saline with 1 mM cobalt chloride and blue marker dye (1 ml), and finally blue marker dye, cobalt chloride, and lymphocytes combined (1 ml) were infused into the vessel specimens. Between each treatment, the integrity of the specimen was verified by flushing with saline solution (1 ml). Vascular permeability was assessed after 15 min of treatment by subjective observation of the extent of extravasation of the blue marker dye onto the surrounding gauze, while qualitative transmigration of lymphocytes was assessed using optical microscopy.

Statistical Analysis

Statistical analysis of data within and between two groups was performed with analysis of variance (ANOVA) for repeated measures followed by Bonferroni's modification of Student's *t*-test. The null hypothesis was rejected when $p < 0.05$. All data are expressed as mean $\pm$ standard error of the mean (SEM). All experiments were performed at least twice, with a minimum of triplicate determinations at each data point. The Bonferroni Dunn posthoc modification for multiple comparisons was used when indicated. An alpha value of $p = 0.05$ was used to assess significance. For all graphs, error bars represent the standard error of the mean, and an asterisk represents a $p = 0.05$.

RESULTS

The Toxicity of the Metal Ions

Toxicity was assessed by measuring LDH release over time. ECs were stimulated by 1 mM Co^{2+} and 1 mM Cr^{3+} over 48 h duration. At the concentrations of metal ions utilized in subsequent experiments, no cellular toxicity was observed as determined by LDH activity at the early time points (Fig. 1).

Dose-Response to Metal Ions on ECs

The dose-response curve for metal ions demonstrated a significant ($p = 0.001$) increase of IL8 (Fig. 2A) and MCP1 (Fig. 2B) after stimulation with 2 mM Co^{2+} and Cr^{3+} . IL8 (Fig. 2A) was also significantly ($p = 0.001$) increased after stimulation with 4 mM Co^{2+} and Cr^{3+} .

Chemokines Release by ECs after Metal Ion Stimulation

Co^{2+} (1 mM) yielded statistically significant increases in both IL8 and MCP1 accumulation in the conditioned media relative to both the NaCl controls as well as to Cr^{3+} peaking after 6 h ($p = 0.001$) or 12 h ($p = 0.001$), respectively. Cr^{3+} did not have a significant effect on the release of either chemokines at any time point (Fig. 3).

Effects of Metal Ions on the Expression of ICAM-1

ICAM-1 was upregulated on ECs after 24 h stimulation with NaCl controls, (Fig. 4A), Co^{2+} (1 mM) (Fig. 4B), Cr^{3+} (1 mM) (Fig. 4C), and as positive control TNF α (Fig. 4D). Total fluorescence revealed statistically significant increases in ICAM-1 upregulation with both Co^{2+} and Cr^{3+} ($p < 0.001$) (Fig. 4E).

Kinetics of ICAM-1 Upregulation by ECs

The timecourse established by Western blot analysis revealed an early and persistent upregulation of ICAM-1 starting at 6 h following the addition of Co^{2+} compared to the other treatment modalities, with continued upregulation through 48 h (Fig. 5).

Metal Ions Enhance Lymphocyte Binding to ECs

Adhesion of lymphocytes on ECs was assessed by two independent assays. Fluorescent binding assay demonstrated significantly ($p = 0.003$) enhanced adhesion of lymphocytes on ECs after 3 h stimulation with Co^{2+} (Fig. 6A). This finding was confirmed by an in vitro binding assay counting adhered lymphocytes (Fig. 6B). Negative controls showed little adhesion of lymphocytes (small oval cells) to the ECs while HUVEC activated with TNF α showed extensive binding. Co^{2+} yielded statistically significant binding of lymphocytes to ECs ($p = 0.002$), while Cr^{3+} did not demonstrate any enhanced binding by the fluorescent binding assay and the in vitro binding assay.

Metal Ions Activate Endothelium Resulting in Lymphocyte Release

Figure 7A depicts control condition demonstrating a sealed vessel. Metal ions alone did not increase vascular permeability despite their ability to activate the endothelium (Fig. 7B). Lymphocytes crossed the activated endothelium when metal ions were present (Fig. 7C).

DISCUSSION

There is a growing body of evidence describing early failures from aseptic loosening of MoM hip replacements, with the tissues surrounding these failed implants being described as pseudotumors. Some of these pseudotumors surrounding failed metal on metal hips have shown collections of lymphocytes and increased vascularity, and had the histological appearance of a Type IV hypersensitivity reaction.^{21,22} In contrast, Campbell et al. showed that some pseudotumors had also features consistent with nonspecific inflammatory reactions to metal wear such as macrophages with particles (similar to those observed around MoP implants). Moreover, the proposed grading system of the pseudotumors like tissues from metal on metal hips standardizes the pseudotumors based upon the histological appearance and how they correlate to the MoM hip replacements.²³ Taken together, the altered local tissue responses might represent a broad spectrum of biologic reactions, as implied by Campbell,²³ with lower concentrations of metal ions producing ALVAL and lymphocyte predominance, while increased wear and the presence of particulate debris generating the more classic histological findings of an osteolytic membrane, with a predominance of macrophages, monocytes, and fibroblasts. Our data suggest that metal ions create distinct biologic reactions, with soluble wear products and the resulting activation of the endothelium as an inciting event. Although previously not described with regard to loosening of implants, the endothelium plays a critical role in inflammatory responses. The interaction of leukocytes with ECs is intrinsic to the process of leukocyte binding and extravasation, whether during the entry of blood polymorphonuclear leukocytes and monocytes into sites of acute and chronic inflammation, or during the homing of lymphocytes to lymphoid organs. One of the key initial steps in lymphocyte migration is attraction through the elucidation of chemokines. Exposure of lymphocytes to the

chemotactic proteins IL8 and MCP1 results in a transition in lymphocytes from circulation to a state of rolling adhesion, along with up regulation of the ligand LFA1. Our results demonstrate that metal ions produced significant increases in the secretion of both IL8 and MCP1, and that at equimolar concentrations, Co^{2+} had a greater effect than Cr^{3+} .

Other studies have described interactions between leukocytes and ECs in response to wear particles.^{24–27} These studies showed an interaction between ECs and leukocytes as well as leukocytes and skeletal muscle cells in response to wear debris from orthopedic implants. However, these studies all evaluated biologic responses to particles, including both metallic debris as well as polystyrene. In contrast, the current study focuses on the effects of soluble metallic ions. Such metallic ions are likely generated at some level with any implants made with components of Co–Cr–Mo alloy, and can be increased to toxic levels under certain circumstances such as component malposition leading to impingement and other abnormal wear. Stable adhesion of lymphocytes to endothelium is crucial for transendothelial migration evoked during inflammatory responses. The basis of stable adhesion involves expression of intercellular adhesion molecule 1 (ICAM-1), an inducible endothelial adhesive protein that serves as a receptor for beta2 integrins on leukocytes. Interaction of ICAM-1 with beta2 integrins enables lymphocytes to adhere firmly to the activated vascular endothelium and subsequently migrate across the endothelial barrier.^{9,28} Our data demonstrate that exposure to either Co^{2+} or Cr^{3+} resulted in the activation of ECs, upregulation of the cell surface binding protein ICAM-1, and enhanced binding of lymphocytes to ECs. While both ions activated the endothelium, Co^{2+} had a greater effect on the upregulation of ICAM-1 compared to Cr^{3+} . Migration and accumulation of lymphocytes into the extravascular space requires activation of the endothelium. Our data shows an activation of the endothelium by metal ions. Activation of endothelium does not necessarily lead to increased vascular permeability. Our data using vessel specimens demonstrated an increase in vascular permeability and lymphocyte extravasation following exposure to metal ions. Metal ions alone activate endothelium but did not increase permeability without lymphocytes present. Interestingly, trafficking of lymphocytes was only seen when vessels were exposed to both metal ions and lymphocytes in combination, again supporting the role of the endothelium as one of the key elements in the formation of ALVAL. Although a number of studies have described the potential biologic effects of Cr^{3+} , including genetic damage, alterations in lymphocyte responses, and even carcinogenesis, our findings suggest that Co^{2+} plays an important role in the generation of ALVAL and ultimately implant failure.^{6,29} In particular, as a divalent cation, Co^{2+} may activate calcium channels, and the addition of Co^{2+} has been shown to induce the transcription factor HIF1 α and increase angiogenesis through the upregulation of VEGF.³⁰ Other studies have shown that Co^{2+} increase lymphocyte binding and transendothelial migration.¹² In our studies, both ions stimulated the upregulation of the EC binding protein ICAM-1, and suggests that the presence of both ions might act synergistically on increasing lymphocyte trafficking.

Although many reports describe both circulating levels of metal ions, local metal levels in the periprosthetic region where particulate debris are generated and are concentrated are less well described.^{7,31,32} However, it would be expected that a concentration gradient of metal ion concentrations would exist, with the highest levels directly adjacent to the bearing surface.³ One study using X-ray emission microanalysis reported significantly higher metal ion concentrations surrounding prostheses compared to serum, urine, or organ levels.¹⁹ Using these findings, local Co^{2+} levels were calculated to be as high as 0.50–1 mM, suggesting that the concentrations utilized in this study were within a clinically significant range.

Co^{2+} ions have been associated with both toxicity as well as allergenic activity,^{11,33} and some studies have suggested that unlike particulate wear debris from metal on polyethylene

articulations, in the case of MoM bearings the release of metal ions also plays an important role in elucidating an undesirable biologic response.³⁴ These concentrations are generally below the limits of the levels defined as a health risk in workers with industrial exposures, but local levels in synovial tissues might easily exceed these levels, and result in local tissue necrosis and adverse biologic reactions. However, the perivascular accumulation of lymphocytes has also been observed following hip replacement with metal on polyethylene articulations.³⁵ Considering that all hip replacements with components consisting of CoMoCr alloys are capable of producing trace levels of these ions, these findings suggest that a threshold effect might exist, and that the generation of pseudotumors may be dose dependent on the levels of metal ions. Given the large surface areas of metal on metal articulations, the bearing surfaces would be expected to generate higher levels of metal ions as seen clinically, and the formation of pseudotumors associated with these implants might reflect a dose dependent biologic reaction to the metal ions as compared to metal on polyethylene articulations. Limitations of the study are the use of Jurkat cells, which are a thoroughly studied immortalized lymphocyte cell line in contrast to fresh isolated lymphocytes.^{10,36,37} Secondly, we did not test metal ions in vivo which would go beyond the scope of this study, which focuses on in vitro effects of metal ions on Jurkat cells and ECs. Taken together, we have shown that the metal ions produced in metal on metal articulations activate the endothelium, and result in increased lymphocyte trafficking and binding. These findings suggest that the endothelium is a central regulatory component in the formation of adverse tissue reactions to metallic wear products, and demonstrate that transendothelial migration of lymphocytes and the perivascular accumulation of lymphocytes in response to metal ions can occur in the absence of sensitized lymphocytes via a Type IV hypersensitivity reaction. Clinically, these alternative mechanisms argue against the routine allergic testing of patients contemplating the use of a metal on metal articulation, and suggest that the responses by the endothelium to metal ions seems to be characteristic of all implants that utilize cobalt chromium molybdenum components. The varying degree is based upon the rate and extent of the generation of these circulating metal ions which seem to play an important role in this process.

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REFERENCES

1. Cuckler JM. The rationale for metal-on-metal total hip arthroplasty. *Clin Orthop Relat Res*. 2005; 441:132–136. [PubMed: 16330995]
2. Antoniou J, Zukor DJ, Mwale F, et al. Metal ion levels in the blood of patients after hip resurfacing: a comparison between twenty-eight and thirty-six-millimeter-head metal-on-metal prostheses. *J Bone Joint Surg Am*. 2008; 3:142–148. [PubMed: 18676949]
3. Cobb AG, Schmalzreid TP. The clinical significance of metal ion release from cobalt-chromium metal-on-metal hip joint arthroplasty. *Proc Inst Mech Eng [H]*. 2006; 220:385–398.
4. Dunstan E, Sanghrajka AP, Tilley S, et al. Metal ion levels after metal-on-metal proximal femoral replacements: a 30-year follow-up. *J Bone Joint Surg Br*. 2005; 87:628–631. [PubMed: 15855362]
5. Dunstan E, Ladon D, Whittingham-Jones P, et al. Chromosomal aberrations in the peripheral blood of patients with metal-on-metal hip bearings. *J Bone Joint Surg Am*. 2008; 90:517–522. [PubMed: 18310701]

6. Jacobs JJ, Urban RM, Hallab NJ, et al. Metal-on-metal bearing surfaces. *J Am Acad Orthop Surg*. 2009; 17:69–76. [PubMed: 19202120]
7. Urban RM, Tomlinson MJ, Hall DJ, et al. Accumulation in liver and spleen of metal particles generated at nonbearing surfaces in hip arthroplasty. *J Arthroplasty*. 2004; 19:94–101. [PubMed: 15578561]
8. Willert HG, Buchhorn GH, Fayyazi A, et al. Metal-on-metal bearings and hypersensitivity in patients with artificial hip joints. A clinical and histomorphological study. *J Bone Joint Surg Am*. 2005; 87:28–36. [PubMed: 15637030]
9. Cook-Mills JM, Deem TL. Active participation of endothelial cells in inflammation. *J Leukoc Biol*. 2005; 77:487–495. [PubMed: 15629883]
10. Hallab NJ, Caicedo M, Finnegan A, et al. Th1 type lymphocyte reactivity to metals in patients with total hip arthroplasty. *J Orthop Surg Res*. 2008; 3:6. [PubMed: 18271968]
11. Jensen P, Thyssen JP, Retpen JB, et al. Cobalt allergy and suspected aseptic lymphocyte-dominated vascular-associated lesion following total hip arthroplasty. *Contact Dermatitis*. 2009; 61:238–239. [PubMed: 19825098]
12. Sultana C, Shen Y, Johnson C, et al. Cobalt chloride-induced signaling in endothelium leading to the augmented adherence of sickle red blood cells and transendothelial migration of monocyte-like HL-60 cells is blocked by PAF-receptor antagonist. *J Cell Physiol*. 1999; 179:67–78. [PubMed: 10082134]
13. Thomas P, Braathen LR, Dorig M, et al. Increased metal allergy in patients with failed metal-on-metal hip arthroplasty and peri-implant T-lymphocytic inflammation. *Allergy*. 2009; 64:1157–1165. [PubMed: 19220218]
14. Kwon YM, Thomas P, Summer B, et al. Lymphocyte proliferation responses in patients with pseudotumors following metal-on-metal hip resurfacing arthroplasty. *J Orthop Res*. 2010; 28:444–450. [PubMed: 19834954]
15. Cai JP, Hudson S, Ye MW, et al. The intracellular signaling pathways involved in MCP-1-stimulated T cell migration across microvascular endothelium. *Cell Immunol*. 1996; 167:269–275. [PubMed: 8603436]
16. Santamaria Babi LF, Moser B, Perez Soler MT, et al. The interleukin-8 receptor B and CXCR chemokines can mediate transendothelial migration of human skin homing T cells. *Eur J Immunol*. 1996; 26:2056–2061. [PubMed: 8814246]
17. Hallab NJ, Jacobs JJ. Biologic effects of implant debris. *Bull NYU Hosp Joint Dis*. 2009; 67:182.
18. Caicedo M, Jacobs JJ, Reddy A, et al. Analysis of metal ion-induced DNA damage, apoptosis, and necrosis in human (jurkat) T-cells demonstrates Ni²⁺ and V³⁺ are more toxic than other metals: Al³⁺, Be²⁺, Co²⁺, Cr³⁺, Cu²⁺, Fe³⁺, Mo⁵⁺, Nb⁵⁺, Zr²⁺. *J Biomed Mater Res A*. 2008; 86:905–913. [PubMed: 18050301]
19. Busse B, Hahn M, Niecke M, et al. Allocation of nonbirefringent wear debris: darkfield illumination associated with PIXE microanalysis reveals cobalt deposition in mineralized bone matrix adjacent to CoCr implants.
20. Wagner M, Klein CL, van Kooten TG, et al. Mechanisms of cell activation by heavy metal ions. *J Biomed Mater Res*. 1998; 42:443–452. [PubMed: 9788508]
21. Davies AP, Willert HG, Campbell PA, et al. An unusual lymphocytic perivascular infiltration in tissues around contemporary metal-on-metal joint replacements. *J Bone Joint Surg Am*. 2005; 87:18–27. [PubMed: 15634811]
22. Pandit H, Vlychou M, Whitwell D, et al. Necrotic granulomatous pseudotumours in bilateral resurfacing hip arthroplasties: evidence for a type IV immune response. *Virchows Arch*. 2008; 453:529–534. [PubMed: 18769936]
23. Campbell P, Ebrahimzadeh E, Nelson S, et al. Histological features of pseudotumor-like tissues from metal-on-metal hips. *Clin Orthop Relat Res*. 2010; 468:2321–2327. [PubMed: 20458645]
24. Kraft CN, Burian B, Diedrich O, et al. Microvascular response of striated muscle to common arthroplasty-alloys: a comparative in vivo study with CoCrMo, ti-6Al-4V, and ti-6Al-7Nb. *J Biomed Mater Res A*. 2005; 75:31–40. [PubMed: 16078208]

25. Kraft CN, Diedrich O, Burian B, et al. Microvascular response of striated muscle to metal debris. A comparative in vivo study with titanium and stainless steel. *J Bone Joint Surg Br.* 2003; 85:133–141. [PubMed: 12585592]
26. Utzschneider S, Becker F, Grupp TM, et al. Inflammatory response against different carbon fiber-reinforced PEEK wear particles compared with UHMWPE in vivo. *Acta Biomater.* 2010; 6:4296–4304. [PubMed: 20570640]
27. Zysk SP, Gebhard HH, Kalteis T, et al. Particles of all sizes provoke inflammatory responses in vivo. *Clin Orthop Relat Res.* 2005:258–264. [PubMed: 15805966]
28. Rahman A, Fazal F. Hug tightly and say goodbye: role of endothelial ICAM-1 in leukocyte transmigration. *Antioxid Redox Signal.* 2009; 11:823–839. [PubMed: 18808323]
29. Delaunay C, Petit I, Learmonth ID, et al. Metal-on-metal bearings total hip arthroplasty: the cobalt and chromium ions release concern. *Orthop Traumatol, Surg Res.* 2010; 96:894–904. [PubMed: 20832379]
30. Liu S, Wu P, Ye D, et al. Effects of lipoxin A(4) on CoCl(2)-induced angiogenesis and its possible mechanisms in human umbilical vein endothelial cells. *Pharmacology.* 2009; 84:17–23. [PubMed: 19478549]
31. Korovessis P, Petsinis G, Repanti M, et al. Metallosis after contemporary metal-on-metal total hip arthroplasty. five to nine-year follow-up. *J Bone Joint Surg Am.* 2006; 88:1183–1191. [PubMed: 16757749]
32. MacDonald SJ. Can a safe level for metal ions in patients with metal-on-metal total hip arthroplasties be determined? *J Arthroplasty.* 2004; 19:71–77. [PubMed: 15578557]
33. Templeton D. Mechanisms of Immunosenitization to metals. *Pure Appl Chem.* 2004; 76:1255–1268.
34. Caicedo MS, Pennekamp PH, McAllister K, et al. Soluble ions more than particulate cobalt-alloy implant debris induce monocyte costimulatory molecule expression and release of proinflammatory cytokines critical to metal-induced lymphocyte reactivity. *J Biomed Mater Res A.* 2010; 93:1312–1321. [PubMed: 19844976]
35. Fujishiro T, Moojen DJF, Kobayashi N, et al. Perivascular and diffuse lymphocytic inflammation are not specific for failed metal-on-metal hip implants. *Clin Orthop Relat Res.* 2011; 469:1127–1133. [PubMed: 21046298]
36. Hallab NJ, Anderson S, Stafford T, et al. Lymphocyte responses in patients with total hip arthroplasty. *J Orthop Res.* 2005; 23:384–391. [PubMed: 15734252]
37. Hallab NJ, Mikecz K, Vermes C, et al. Differential lymphocyte reactivity to serum-derived metal-protein complexes produced from cobalt-based and titanium-based implant alloy degradation. *J Biomed Mater Res.* 2001; 56:427–436. [PubMed: 11372061]

Figure 1.

Cellular toxicity was measured by LDH release from ECs after ion stimulation. Metal ions induced LDH release after 48 h. No cellular toxicity was observed at earlier time points. TNF α is the control.

Figure 2.

A: IL8 ($p < 0.001$, †, control compared to 2 mM Co^{2+} and 4 mM, respectively) and (B) MCP1 ($p < 0.001$, †, control compared to 2 mM) release is significantly induced as measured by Student's ttest after 8 h stimulation with Co^{2+} but not with Cr^{3+} as measured by ELISA in the conditioned media.

Figure 3.

A: IL8 and (B) MCP1 release from ECs after stimulation with Co^{2+} (1 mM) and Cr^{3+} (1 mM) measured by ELISA in conditioned media. Stimulation with Co^{2+} over 30 h significantly ($p < 0.001$ *) vs. control at the same time point) increased as measured by Student's ttest except at the earliest time points. Cr^{3+} did not have an effect.

Figure 4. ICAM-1 expression was significantly (all # vs. control, p values <0.001 assessed by Bonferroni) increased in ECs after stimulation with Co²⁺ (1 mM) and Cr³⁺ (1 mM) ions as assessed by immunohistochemistry depicted as green granular labeling of the membrane and blue nuclei. A. is control without stimulation, B. is Co²⁺ 48 h stimulation, C. is Cr³⁺ 48 h stimulation, D. depicts TNFα as positive control, E. depicts fluorescence intensity.

Figure 5.

A significant increase in ICAM-1 expression (# vs. control, $p < 0.001$ at all time points) in ECs stimulated with Co^{2+} (1 mM) was measured by western blot. Cr^{3+} did not increase ICAM-1 expression significantly at any time point. $\text{TNF}\alpha$ served as control. A. depicts an autoradiograph; in B. a bar graph depicts band densities.

Figure 6.

(A and B) Stimulation with Co²⁺ (1 mM) induced lymphocyte adhesion to ECs in two different adhesion assays. A. measures fluorescence intensity by plate reader ([#], Co²⁺ vs. control, $p = 0.003$); B. adhering lymphocytes were counted ([#], Co²⁺ vs. control, $p = 0.002$). TNF α served as a positive control.

Figure 7. Rabbit carotid artery without blue dye and Co^{2+} is depicted in A. In situ permeability assay demonstrated that cobalt ions and blue dye alone did not increase permeability as seen B. After addition of lymphocytes the blue dye and the lymphocytes leaked out of the carotid artery suggesting increased vascular permeability (C).